

CONTRAST MEDIA NEUROTOXICITY

In vitro and in vivo studies of intrathecal
contrast media toxicity using rat and rabbit
models with a clinical correlation

Sven E. Ekholm
leg. läk., Helsingkrona



Lund 1984

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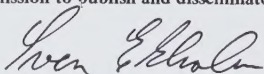
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Abstract The aim of the investigation was to study some factors that might explain the origin of the adverse reactions that sometimes develop following subarachnoid investigations with metrizamide. The presence of a glucose analogue in its molecule has given rise to speculations about metabolic disturbances and an in vitro model using rat neural tissue was designed to examine the influence of metrizamide on neural tissue glucose metabolism. The hypothesis that metabolic interference was the result of the molecular structure was tested by comparing metrizamide to a new nonionic contrast medium, io-hexol, which has no glucose analogues in its molecule. Besides the pharmacologic properties of a contrast medium, toxic effects are also related to tissue concentration and contact time with the neurons. Therefore, a rabbit model was designed to study the pharmacokinetics of metrizamide in neural tissue following lumbar myelography and compare it to iohexol. The clinical significance of these experimental findings was evaluated in a clinical trial comparing metrizamide to iohexol. Conclusions from the investigation are that: 1. The molecular structure of metrizamide may interfere with neural tissue metabolism. 2. The iohexol molecule does not interfere with neural tissue metabolism. 3. There are indications that metrizamide is partially trapped within neural tissue following in a prolonged contact time with neurons. 4. Iohexol follows a simple diffusion model for neural tissue penetration. 5. Iohexol is superior to metrizamide with respect to adverse reactions.			
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This thesis is based on the following papers which will be referred to in the text by their Roman numerals:

- I. Ekholm SE, Reece K, Coleman JR, Kido DK and Fischer HW: Metrizamide - a potential in vivo inhibitor of glucose metabolism. Radiology 1983: 147: 119 - 121
- II. Ekholm SE, Reece K, Foley M, Kido DK and Morris TW: The effect of iohexol on glucose metabolism compared to metrizamide. Invest Radiol 1984 (In press)
- III. Ekholm SE, Foley M, Kido DK and Morris TW: Metrizamide lumbar myelography in rabbits - A study of contrast media penetration and resorption. Acta Radiol Diagnosis 1984 (Accepted for publication)
- IV. Ekholm SE, Foley M, Morris TW and Sahler L: Neural tissue uptake and clearance of iohexol in rabbit myelography. Acta Radiol Diagnosis 1984 (Accepted for publication)
- V. Hindmarsh T, Ekholm SE, Kido DK, Sahler L and Sands M.: Iohexol and metrizamide lumbar myelography: A double-blind clinical trial. Acta Radiol Diagnosis 1984 (Accepted for publication).

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Until the introduction of computerized tomography and more recently magnetic resonance imaging radiographic evaluation of the spinal cord and the subarachnoid space was not possible without an intrathecal injection of contrast medium. The attenuation differences between the neural tissue, cerebrospinal fluid (CSF) and surrounding soft tissue structures are too small to allow their visualization with conventional radiography alone. It is possible to better define these structures with computerized tomography but in many cases there is still a need of a subarachnoid contrast medium to increase visualization of the spinal cord.

Gas was the first contrast medium used in the subarachnoid space to allow visualization of the cord as well as certain intracranial structures /1 - 3/. The commonly used gases (air, oxygen and nitrous oxide) had the advantage of being nontoxic to the central nervous system (CNS) and completely absorbed following the examination. However, in the spinal canal gas was mainly used for localization of mass lesions and was not adequate for the definition of fine structures such as nerve roots and blood vessels. In view of this diagnostic limitation as well as causing some rather severe side effects due to disturbed pressure balance from the exchange of CSF for gas, the need for a positive contrast medium was evident.

The first positive contrast medium used in the subarachnoid space was an iodized poppy seed oil /4/ which later was followed by another oil-based contrast medium, iophendylate /5/. Iophendylate has very few immediate adverse effects but it is not cleared from the subarachnoid space to a significant degree and can cause chronic inflammatory changes if it is not removed following the examination /6/. However, there are some important diagnostic disadvantages with the use of iophendylate. It is an oil-based contrast medium that will not mix with CSF and the high viscosity results in poor filling of the root sleeves. Moreover, the radiographic density is very high which makes visualization of fine details difficult.

In 1931 the first water soluble contrast medium for subarachnoid investigations, sodium methiodal, was introduced /7/. As a water soluble contrast medium, sodium methiodal had some diagnostic advantages; a lower viscosity that allows better definition of nerve root sleeves, it mixes with CSF and provides a lower density thus improving the demonstration of finer details. Moreover, since it drains into the blood along with CSF it is not necessary to remove it following the investigation. Sodium methiodal, however, is very irritating which necessitated spinal anesthesia and hindered its use above the lumbar region. From a practical and diagnostic viewpoint it was obvious that a nontoxic water soluble contrast medium should be the agent of choice and several attempts were made to develop a better contrast medium. It was not until 1964 with the introduction of meglumine iothalamate for lumbar myelography that some improvement was achieved /8/. This contrast medium could be used without supplementary spinal anesthesia but it was still too toxic for use above the lumbar region. A further reduction in toxicity was achieved with meglumine iocarmate, introduced in 1969 /9/ but the neurotoxicity was still significant and even this contrast medium had to be restricted to the lumbar region.

So far, all these water soluble contrast media had been ionic agents with problems of high osmolality and free salt-forming ions that interfered with the normal neural function. The first major improvement was seen in 1972 with the introduction of a nonionic water soluble contrast medium, metrizamide (Amipaque®). This contrast medium was the result of a suggestion by Almén in 1968 that nonionic contrast media without salt-forming ions would reduce the osmolality compared to ionic contrast media with identical iodine concentration /10/. Metrizamide proved to be far less neurotoxic than previous contrast media and it became the first water soluble contrast medium generally used for investigations throughout the entire subarachnoid space. However, metrizamide is not completely devoid of neurotoxic properties and it has been accompanied by a number of adverse reactions. Most of these are minor, such as headache and nausea, but there have also been a significant number of more serious reactions, such as mental disturbances and seizures /11 - 14/. Another disad-

vantage of metrizamide is that it is supplied as a lyophilized powder which makes it inconvenient to use since it has to be dissolved immediately before the examination. The idea of a nonionic water soluble contrast medium was established and metrizamide is now being followed by the second generation of nonionic contrast media, one of which is iohexol (Omnipaque^R). Experimental /15 - 16/ and clinical tests /17 - 18/ have been encouraging and iohexol was registered in Sweden for lumbar myelography in 1984.

Aim of the Investigation

The aim of the present investigation was to study some factors that might explain the origin of adverse reactions which sometimes develop following subarachnoid investigations with metrizamide. A better understanding of the etiology of adverse effects should be helpful in predicting factors that may predispose to adverse reactions with future contrast media.

Metrizamide is almost isotonic in concentrations commonly used for lumbar myelography and being nonionic it has no free radicals which can explain its neurotoxic effects. It has been speculated that the adverse effects seen following the use of metrizamide are a result of disturbances in neural tissue metabolism. The central nervous system is almost exclusively dependent upon D-glucose for its metabolism and interference in glucose metabolism will result in neural dysfunction. Some D-glucose analogues, such as 2-deoxy-D-glucose (2-DG), compete with D-glucose for the intracellular transport mechanism as well as for certain intracellular enzymes. It is therefore tempting to assume that the adverse reactions following metrizamide myelography is a result of such a metabolic disturbance since 2-DG is a part of the metrizamide molecule (Fig. 1). The new and apparently less toxic nonionic contrast media, one of which is iohexol (Fig. 1), are devoid of glucose analogues in their molecules and thus should not interfere with glucose metabolism. In view of this an experimental model was developed:

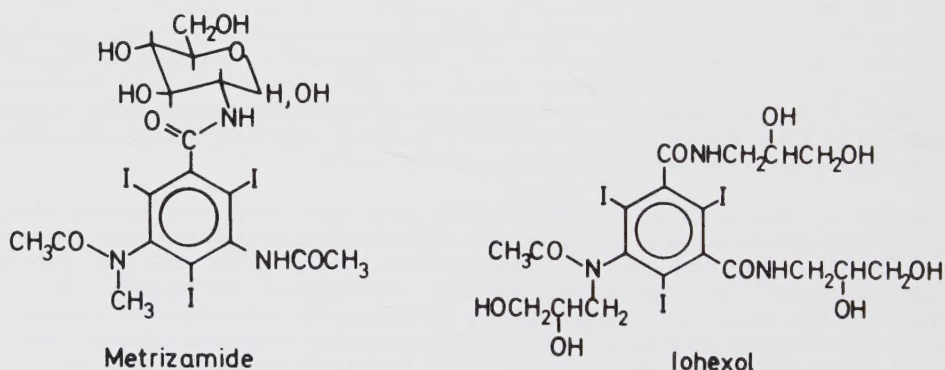


Fig. 1. Molecular structure of metrizamide and iohexol.

- . to study the in vitro effect of metrizamide on glucose metabolism in neural tissue (I).
- .. to investigate if iohexol is devoid of metabolic disturbances (II).

Besides the pharmacologic properties of a contrast medium, toxic effects can also be related to tissue concentration and contact time with the neurons.

Therefore, it was decided:

- . to develop an animal model for lumbar myelography and investigate the pharmacokinetics of neural tissue diffusion for metrizamide (III).
- .. to investigate if iohexol differs from metrizamide in its pharmacokinetics for neural tissue diffusion (IV).

Differences in toxicity seen in animal experiments are supportive but not directly transferable to the clinical situation. To evaluate the significance of findings from the animal experiments it was decided:

to perform a double-blind clinical trial comparing metrizamide and iohexol in lumbar myelography and evaluate if the differences in toxicity seen experimentally are pertinent in clinical practice (V).

Summaries of Material, Methods, and Results

- A. Experimental investigations of in vitro effects of metrizamide and iohexol on glucose metabolism in neural tissue.

Methods Used in Investigations I - II

Hippocampal tissue from adult Wistar rats were used for these experiments. The hippocampi were removed from the rat brain in an unaltered state following decapitation. Each hippocampus was sectioned perpendicular to the long axis into 250 μ m thick slices using a TC-2 tissue sectioner (Sorvall). Following sectioning, the tissue slices were placed in an artificial CSF-medium-124 mM NaCl, 2.0 mM KCl, 1.25 mM KH_2PO_4 , 2.0 mM MgSO_4 , 26 mM NaHCO_3 and 5 mM D-glucose. Twenty tissue sections from a single hippocampus were then divided into two groups. Each group of 10 was placed in a separate tube containing 4 ml CSF medium. One of the groups had 1.2 ml of test substance added and the other group was used as a control. The control was either nondiluted or had 1.2 ml of a control substance added to the CSF medium. The CSF medium was bubbled with 95 % O_2 and 5 % CO_2 after which the tubes were sealed and placed in a shaker bath at 37.5° C (Fig. 2).

Following 4 hours of incubation another 2 ml CSF medium containing ^{14}C -labelled D-glucose was added to each vial. Tubes containing test or control media also had another 0.6 ml of the test or control medium added to maintain their concentrations. Bubbling with O_2/CO_2 of the CSF medium was repeated and the tubes were replaced in the shaker bath.

Shaker Bath

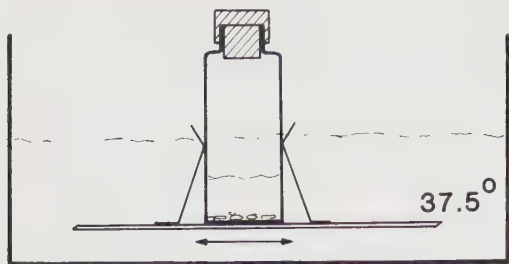


Fig. 2. The incubation of tissue samples in a shaker bath.

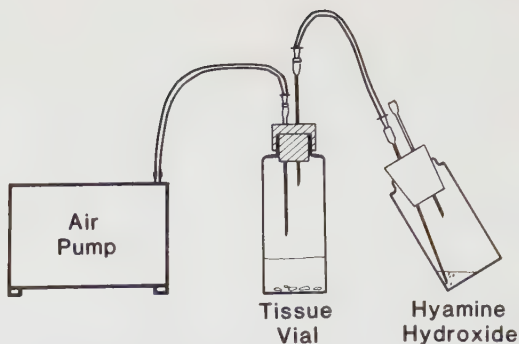


Fig. 3. System for collecting metabolically produced $^{14}\text{CO}_2$.

Two hours later metabolism was stopped by injecting 1.0 ml of 2N HCl thereby also releasing the buffered CO_2 in the CSF medium. The released CO_2 , including the metabolically produced $^{14}\text{CO}_2$, was collected in a separate vial containing 1 ml of a strong basic solution, hyamine hydroxide, which binds CO_2 (Fig. 3). Following the CO_2 collection 10 ml of Ultrafluor Scintillation Fluid was added to the hyamine hydroxide and the $^{14}\text{CO}_2$ content was measured in a Beckman 8100 automatic liquid scintillation counter. The amount of tissue used for each sample may vary slightly which is why the relative amount of tissue in each tube was determined with a spectrophotometric protein assay (Bio-Rad Lab). Since the tissue in each paired sample originated from a single hippocampus, it was assumed that tissue viability was identical and metabolic activity was expressed as disintegration per minute per μg of protein.

The reason for measuring the CO_2 production for only the last 2 hours out of 6 was to secure the detection of a metabolic disturbance independent of the cellular level of interference. Metabolic interference would most likely take place extracellularly at the glucose carrier level since intracellular localization of metrizamide has never been proven. However, if there is an intracellular enzymatic effect one might assume that a certain time should be needed to reach a metrizamide concentration high enough to demonstrate an effect on metabolism.

The mean difference in CO_2 production of all paired samples in the study was considered significant when p-values < 0.05 were obtained (Student's t-test for paired samples).

Metrizamide - a Potential in vivo Inhibitor of Glucose Metabolism (I)

Material and Methods:

Sixteen paired hippocampal tissue samples were used to evaluate the effect of isotonic metrizamide (170 mg I/ml) on glucose metabolism compared to a non-diluted control.

The addition of metrizamide causes a dilution of the CSF medium. To evaluate the influence of this, metrizamide was replaced with an isotonic polyethylene glycol (MW = 400 daltons) solution in another 16 paired samples. The osmolality of the solution was determined by the freezing point technique.

Results:

The addition of isotonic metrizamide to the CSF medium resulted in a mean reduction in CO₂ production of 24.4 % ($\pm$ 4.78 SEM) compared to the nondiluted controls. This decrease in CO₂ production in the presence of metrizamide was significantly lower than that of the controls ($p < 0.001$).

Replacement of metrizamide with polyethylene glucol demonstrated a mean increase in CO₂ production in the polyethylene glucol samples of 16.9 % ($\pm$ 14.3 SEM) compared to the non-diluted controls but this was not significant.

The Effect of Iohexol on Glucose Metabolism Compared to Metrizamide (II).

Material and Methods:

Eighty paired samples were used for these experiments.

Primarily iohexol was compared to metrizamide at 180 mg I/ml, which is a commonly used concentration of iohexol for lumbar myelography. Iohexol in this concentration is slightly hypertonic, 415 mOsm per kg water. Isotonic iohexol (140 mg I/ml) was therefore tested against iohexol at 180 mg I/ml and against isotonic metrizamide (170 mg I/ml). Finally, to test if a more marked hyperosmolality could have any impact on metabolism, another study was also performed comparing iohexol at 240 mg I/ml (500 mOsm per kg water) to metrizamide at 180 mg I/ml as well as to a non-diluted control.

The osmolality of the solutions was determined by the freezing point technique.

Results:

The comparison of iohexol and metrizamide at 180 mg I/ml showed a 23.3 % ($\pm$ 11.1 SEM) higher CO₂ production for the iohexol containing samples. This CO₂ production in the iohexol samples was significantly higher than that of the metrizamide samples ($p < 0.05$). The comparison of isotonic iohexol and iohexol at 180 mg I/ml showed only a 7.8 % ($\pm$ 6.5 SEM) higher CO₂ production for the isotonic samples, which was not statistically different. When iohexol and metrizamide were compared to each other in isotonic concentration the CO₂ production was 27.4 % ($\pm$ 9.9 SEM) higher for the iohexol samples ($p < 0.02$). This difference is slightly larger than that of the initial study when the two contrast media were compared at 180 mg I/ml. This seems to indicate a mild depressive effect caused by the hyperosmolality of iohexol since the difference in osmolality of metrizamide at 170 versus 180 mg I/ml is very small.

When a more hypertonic solution of iohexol, 240 mg I/ml, was compared to metrizamide at 180 mg I/ml the iohexol samples demonstrated a 17.8 % ($\pm$ 7.6 SEM) lower CO₂ production than the metrizamide samples. This difference was significant at a $p < 0.05$ level. In the comparison of iohexol at 240 mg I/ml to a non-diluted control the CO₂ production was 61.2 % ($\pm$ 4.3 SEM) lower in the iohexol samples ($p < 0.001$).

These combined data failed to show any evidence of metabolic disturbances caused by the iohexol molecule. There is, however, a depressive effect of hyperosmolality in vitro which is more pronounced than expected from a linear relationship.

B. Experimental in vivo investigations of pharmacokinetics of contrast media diffusion in neural tissue.

Methods Used in Investigations III - IV

New Zealand white rabbits of both sexes were used for these experiments. The animals were divided into four groups which varied in investigation time; 1, 2, 4 and 8 hours. All of these animals had 0.5 ml of a contrast medium, metrizamide or iohexol, injected into the lumbar subarachnoid space. Both contrast media were used in a concentration of 180 mg I/ml.

There was also one control group in each study which had the contrast medium replaced with an inert isotonic solution. The animals were continuously monitored for blood pressure, heart rate and body temperature. Blood gases were controlled regularly. To secure subarachnoid localization of the contrast medium a lumbar laminectomy was performed and a polyethylene catheter (PE 10) was inserted into the subarachnoid space. The catheter tip was positioned at the level of vertebrae L 7 - S 1 and the site of insertion in the dura was sealed with cyanoacrylate. Before injection of the contrast medium the cranial end of the restraining board was elevated 30 - 35° and kept in this position throughout the entire experiment to avoid gravitational flow of CSF/contrast medium. The localization of the contrast medium was verified radiographically.

Upon completion of the experiment the animals were sacrificed and 0.2 ml of CSF samples were obtained from the suboccipital and lumbar regions for determination of their contrast medium content. For evaluation of neural tissue diffusion, samples were taken for contrast medium determination from the cervical, mid-thoracic and lumbar cord. The samples were analyzed in an X-ray excitation fluorescence spectrophotometer and the iodine content was expressed as mg I/g of tissue or ml of CSF.

Metrizamide Lumbar Myelography in Rabbits - A Study of Contrast Media Penetration and Resorption (III)

Material and Methods:

Twenty-nine rabbits weighing between 2.5 and 4.8 kg were used in this study. The one and 2 hour groups consisted of 7 animals each and there were 6 animals in each of the 4 and 8 hour groups. These 26 rabbits received 0.5 ml metrizamide and the other 3 rabbits were used as controls.

Results:

The CSF data demonstrates a rather large interanimal variability within each group. This is probably a result of differences in the production rate of CSF which could influence the drainage of CSF/contrast medium to the blood. The overall trend, however, shows a decrease in lumbar CSF iodine over time as expected (III: Fig. 3). The iodine concentration in the suboccipital CSF is generally much lower than in lumbar CSF and 11 animals had no detectable iodine (III: Fig. 4). These data indicate that cranial flow of lumbar CSF is very low in this rabbit model. The same pattern can be demonstrated by looking at the concentration of iodine in the spinal cord at different levels (III: Tables 1 and 2). The bulk drainage of lumbar CSF in this model thus seems to take place in the lumbosacral region.

In contrast to the decrease in lumbar CSF the iodine concentration in the lumbar cord seems almost unchanged with time for up to 8 hours (III: Fig. 5). This apparent delay in clearance of metrizamide in neural tissue is indicative of some retention of metrizamide in the tissue since a more parallel decrease in the iodine concentration

would be expected in lumbar CSF and cord if tissue clearance was by simple diffusion. However, the large interanimal variability necessitates a direct comparison of lumbar CSF and cord concentrations for each animal. The immediate impression when the animal data are plotted on a graph in this way is that of a simple diffusion with data gathered along a common regression line (III: Fig. 1). However, when the time of sacrifice is added as a covariant, each group of animals appears to have a separate regression line, the one hour group being most widely separated from the 8 hour group (III: Fig. 2), which indicates some retention of metrizamide in the tissue. The difference in the intercepts of the four regression lines was tested with the Bonferroni method of multiple comparisons at an overall level of significance of 0.05. The 8 and one hour lines differed significantly while the others were not significant.

Neural Tissue Uptake and Clearance of Iohexol in Rabbit Myelography (IV)

Material and Methods:

Twenty-eight rabbits weighing between 3.6 and 5.6 kg were used for this study. There were 6 animals in each of the 4 groups which received iohexol. Four animals acted as controls.

Results:

The lumbar CSF concentration of iohexol decreases with time similarly to metrizamide as described in the previous paper (IV: Fig. 3). The cranial flow of CSF/contrast medium from the lumbar region was even less than that shown in the metrizamide study (IV: Fig. 3), but there is probably no true difference between the two contrast media. There is, however, a difference in clearance of the two contrast media in the lumbar cord (Table I). Iohexol decreases in concentration with time (IV: Fig. 4) along with the decrease in lumbar CSF indicating a simple diffusion of iohexol in neural tissue. When the lumbar CSF and cord concentration was plotted for each animal no significant difference could be seen between the groups (Bonferroni method of multiple comparisons). Iohexol, in contrast to metrizamide, thus shows no indication of neural tissue retention.

	Mean iodine concentration (mg I/g tissue)							
	1 hour		2 hours		4 hours		8 hours	
Metrizamide	1.45	$\pm$ 0.44 SEM	0.83	$\pm$ 0.33 SEM	1.36	$\pm$ 0.59 SEM	1.91	$\pm$ 1.12 SEM
Iohexol	2.33	$\pm$ 0.35 SEM	2.33	$\pm$ 0.76 SEM	1.99	$\pm$ 0.59 SEM	0.42	$\pm$ 0.11 SEM

TABLE I. Clearance of iodine in the lumbar cord with time for metrizamide and iohexol.

Iohexol and Metrizamide Lumbar Myelography: A Double-Blind Clinical Trial (V)

Material and Methods:

The number of patients, their sex, age and injected volume are shown in Table II. The study involved patients referred for lumbar myelography with a suspicion of either a herniated disc or spinal stenosis. The patients, 18 years or older, entered the study consecutively provided they were not excluded based on the criteria listed (V: Table I).

	Number of patients		Age	Dose injected (ml)
	Males	Females	Mean (range)	Mean (range)
Iohexol (180 mg I/ml)	12	13	47.5 (18 - 79)	16.12 (11 - 17)
Metrizamide (180 mg I/ml)	15	10	42.6 (18 - 64)	16.38 (12 - 17)

TABLE II. Clinical trial of metrizamide and iohexol. Summary of patient data.

The contrast medium assigned to each patient was determined by a computer generated randomized code to which only the assistant who prepared the agent had access. The examination included an AP view of the conus region but the contrast medium was never allowed to reach higher than the tenth thoracic vertebra. Post-procedural care were identical for all patients with rest for 24 hours with the head of the bed raised 30°. Restroom privileges were allowed 8 hours after the examination. The tests performed to evaluate adverse reactions are listed in Table III. A neurological examination was performed by two trained neurosurgeons.

	Before myelography	After myelography				Succeeding days
		0-4 hrs	4-6 hrs	6-10 hrs	22-24 hrs	
Subjective symptoms experienced by the patient		X		X	X	Only when persistent symptomatology
Routine blood analysis	X				X	
CSF analysis		X (sampled before inj. of contrast medium)				
Neurological exam	X		X		X	

TABLE III. Summary of tests and examinations performed in the clinical trial.

For comparison two other patient groups were also questioned for adverse reactions in the same way. One of these groups was comprised of 20 patients (10 males and 10 females) with suspected cervical disc herniation who were examined with iophendylate (Pantopaque^R). These patients had the same preparation and post-myelographic regimen as the patients in the double-blind trial. The other group consisted of 20 patients (10 males and 10 females) who only had a lumbar puncture as part of a neurological work-up.

The image quality was assessed by an independent radiologist.

Results:

The subjective adverse reactions reported by the patients are summarized (V; Fig. 1). The most commonly reported adverse reaction among the patients in the double-blind trial was headache which was more common among patients who had been studied with metrizamide (15 cases) than iohexol (9 cases). Nausea was experienced by 4 patients who received metrizamide and 5 of the iohexol patients. This was always combined with a headache. Persistent headache, exceeding 24 hours and not relieved by drugs, was recorded in one patient who had metrizamide and 2 iohexol patients. One of the latter had both headache and nausea for 3 days following the myelography but this patient was later thought to have emotional problems. Aggravation of preexisting back pain or sciatica, which is a well-known complication of myelography in patients with lumbar disc herniation, occurred in 2 patients following iohexol myelography. Clinical and laboratory examinations did not disclose any obvious changes.

Discussion

The central nervous system (CNS) appears to require a very stable environment to function properly which involves several control mechanisms for its protection /19 - 20/. The concentration of various ions and non-electrolytes in the cerebrospinal fluid (CSF) and the extracellular space of the CNS differs somewhat from those in serum /21/. Most significant is the lower concentration of protein, glucose and potassium, and the higher concentration of magnesium. The two ions, potassium and magnesium, have significant effects on the excitability of neurons and it may well be that their particular concentrations hold excitability at a lower level than if the neurons were surrounded by a plasma filtrate. The composition of CSF thus largely has to depend on specific transport mechanisms /22/ and many water soluble solutes in the blood like contrast media will either not pass the brain capillaries or enter very slowly /23/. Contrast media injected into the subarachnoid space will by-pass this barrier and may interfere with the delicate ion balance necessary to maintain a normal neural function /24/.

Water soluble contrast media mix with CSF and are transported out of the subarachnoid space along with CSF. This absorption to the blood is largely regulated by the production rate of CSF. Most of the CSF formation takes place in the ventricular system of the brain and the major portion is normally derived from the choroid plexus. Experiments with isolated choroid plexus preparations indicate that 80% or more of CSF is produced from this source alone /25, 26/. The parenchyma of the CNS is probably the principal source of extrachoroidal CSF /27, 28/. Support for this is found in several studies which have shown that extracellular markers introduced into the parenchyma move towards the surface of the brain, at a rate which is independent of their molecular weights, indicating a flow of fluid in this direction /27, 28/. The mechanism of CSF production is complex and the current explanations largely speculative /29 - 31/. In the first phase an ultrafiltrate of plasma passes through the capillary endothelium of the choroid plexus by hydrostatic pressure into the surrounding interstitial tissue. This filtration can occur because the capillaries of the choroid plexus lack tight junctions. Further transport, at least for larger molecules, is hindered by the tight junctions between the epithelial cells covering the choroid plexus /32/. The further transport of ions from the interstitial tissue to the CSF is partially dependent upon an intracellular carbonic anhydrase catalyzation and an active sodium-potassium pump. The transfer of chloride ions may either be coupled to this pumping process or there may be a separate transport system /22, 33/. It is assumed that the local osmotic forces created are responsible for the

movement of water which is indicated by the negligible difference in the osmolalities of plasma, plasma ultrafiltrate and CSF /19/. Substrates for CNS metabolism such as D-glucose and L-amino acids, are transported over the blood-brain barrier by specific carriers in the cell membranes /34 - 35/. There are several possibilities for interference with this complex system of CSF production which secondarily could influence the clearance of contrast medium and other substances in the CSF. Intravascular injection of certain drugs, for example acetazolamide /36/ and furosemide /37 - 38/, have been shown to reduce CSF formation markedly in animal experiments. This reduction in CSF production by acetazolamide has also been demonstrated in man /39/. Other factors which may interfere with CSF production are rapid changes in serum osmolality caused by fluid injection and changes in the acid-base balance of serum. Rapid increase in serum osmolality in animals results in reduced CSF formation while acute decrease in osmolality will have the opposite effect /40/. It has also been suggested in an animal model that dehydration may decrease the absorption rate of CSF/contrast medium following myelography and thus increase the risk of toxic effects from the contrast medium /41/. With respect to disturbances in the acid-base balance, alkalosis is the only known cause which has any significant influence causing a decrease in CSF production /42/. This effect is most pronounced for respiratory alkalosis and is probably the result of a decrease in the substrate(carbon dioxide) for carbonic anhydrase activity. Thus changes in CSF production due to acid-base imbalance should not have been a problem in the studies of diffusion kinetics since none of the rabbits had alkalosis or a significant drop in pCO_2 (III - IV). Neither should dehydration be an important factor since the animals had free access to water prior to the study and fluid losses during the experiment were compensated for by intravenous injections of an isotonic solution (III - IV). Osmolality controls on a few animals also support this assumption. If differences in CSF production were the cause of the large interanimal variation in contrast medium absorption, this may simply reflect a normal interanimal variation.

Contrast media themselves may also interfere with CSF production. Harnish et al /43/ were able to demonstrate a reduction in CSF production following intravenous injection of an ionic contrast medium and more recently with a non-ionic contrast medium, iohexol (unpublished data). The choroid plexus is innervated by both adrenergic /44/ and cholinergic /45/ fibers. Stimulation of the adrenergic fibers will diminish CSF flow by about 30 % acutely and denervation results in an increase of the flow of approximately 30 % /44/. The cholinergic innervation has the opposite effect to the adrenergic fibers and stimulation of the cholinergic fibers will increase CSF flow by as much as 100 % without changes in the blood flow to the choroid plexus /46/. In an invertebrate preparation Marder et al /47/ demonstrated that metrizamide acts both as an antagonist of cholinergic transmission and an inhibitor of the enzyme acetylcholinesterase. Dawson /48/ showed a relative inhibitory effect of cholinesterase from both ionic and non-ionic contrast media, least pronounced with iohexol, and this effect may be the cause of reduction in CSF flow as seen following intravenous injection of contrast media /43/.

The only force shown to be responsible for absorption of CSF to the blood is that of a hydrostatic gradient with the absorption rate being relatively linear over a rather large physiological range /39, 49 - 50/. When pressure is raised above normal physiological levels the resistance to flow also appears to diminish /51/. The absorption of CSF under normal physiological conditions has until quite recently been regarded as taking place over the arachnoid granulations and villi alone. Indications of a supplementary lymphatic drainage in earlier investigative work have been largely ignored /52-53/. The arachnoid villi and granulations seemed ideally situated to drain CSF from the subarachnoid space into the major dural sinuses. The importance of these structures in CSF drainage has also been well established /52 - 54/. Smaller arachnoid villi granulations also exist along the spinal nerve roots /55/. There has been some controversy about how CSF is drained through the granulations and villi. Some have favoured a closed channel theory with creation of a transendothelial vacuolization process /56/ and others an

open endothelial lined channel system /30, 57/. Levine et al /58/ were unable to show the existence of intracellular vacuoles or apical pores at negative pressure gradients. With an increase in the pressure gradient, the number and size of vacuoles increased, as did the number of pores. The diameter of these openings were never more than 1 - 2 μ m. Most of the interendothelial junctions were tight junctions but some were of the open variety at every pressure. Isolated flux chamber studies of the drainage pathways in primate arachnoid villi showed that microspheres with a diameter below 2 μ m as well as intact red blood cells, 7.5 μ m, passed through the villi /59/. The red blood cells of man are known to pass through channels as small as 2.9 μ m in diameter without hemolysis /60/ and this may indicate that some channels in primates are even larger than 2 μ m. Recent studies have shown that CSF is also normally drained via prelymphatic pathways through the cribriform plate to the cervical lymph nodes /61 - 62/. It seems reasonable, although not yet shown, that this kind of lymphatic drainage may also take place in other regions including the spinal canal. Bradbury et al /62/ demonstrated that a substantial amount of CSF is absorbed via these prelymphatic pathways out through the cribriform plate and that the magnitude of the flow was subject to hydrostatic and postural factors. This pathway may be important especially for larger molecules but the rate of recovery in lymph of molecules of a molecular weight of 5000 daltons and below was insignificant /62/. This appears to indicate that this pathway normally is only of minor significance for the absorption of water soluble contrast media, such as metrizamide and iohexol, since their molecular weight is less than 1000 daltons.

The CSF volume in an adult man is about 150 - 200 ml and is replaced approximately three times a day. The cerebrospinal fluid produced in the ventricles is transported out of the ventricular system by pulsatile volumetric changes of the brain and the vessels of the choroid plexus corresponding with each cardiac cycle /63/. Cerebrospinal fluid is also propelled towards the fourth ventricle and out of the ventricular system by currents produced by the ependymal cilia /64/. There is a downward, gravitational flow of CSF along the spinal cord and CSF will reach the lumbar sac in 60 - 90 minutes as measured by tracers injected into cisterna magna /65/. There are also some indications for cranial circulation of lumbar CSF. Labelled albumin injected into the lumbar subarachnoid space could be traced in the basal cistern after approximately one hour, reaching the intracranial cavity in about 12 hours /66/. Studies of CSF absorption following lumbar myelography in rabbits proved that most of the contrast medium is absorbed in the lumbar area and very small amounts reached the cisterna magna /67/. These findings are in accordance with the results of the present study (III - IV). There are some differences between man and rabbits in CSF flow dynamics and a different absorption pattern might be expected in man. There are some indications that contrast medium in man is absorbed in the lumbar region following lumbar myelography. In normal RIHSA cisternograms, with the tracer injected into the lumbar subarachnoid space, it will take 10 - 12 hours before the tracer is distributed over the cerebral subarachnoid space /68/. Thus it seems reasonable to believe that a large portion of the contrast medium is absorbed, in the spinal region since the biological half-life of subarachnoid metrizamide in man has been calculated to be approximately 4 hours /69/.

The extracellular space of the CNS was for a long time generally considered to be very small and insufficient to serve as a nutritive media for the neurons. This gave rise to the hypothesis that the astrocytic foot serves as some kind of nurse cell for the neurons. Later studies have shown that the volume of the CNS extracellular space is almost identical to that of other tissues in the body. In rabbit, cat, dog and monkey, it has been estimated to be approximately 15 - 18 % in gray matter and 12 - 13 % in those regions which contain a large proportion of white matter such as the medulla and spinal cord /70/. The amount of extracellular fluid appears to be sufficient to serve as a transport medium for the neurons. The reason for the initial underestimate of the extracellular space was the sink function of CSF and more accu-

rate values have been shown by keeping the concentration of the tracer identical in serum and CSF /71/. In contrast to the barrier between blood and CNS there is apparently no barrier between the CSF and CNS. The cells of the pia mater and ependyma are joined to each other by gap junctions which will allow a free exchange of water soluble substances between the CSF and extracellular space of the CNS /32, 34, 72 - 73/. There are a few exceptions, for example the epithelial cells covering the choroid plexus are joined by tight junctions similar to those seen in the endothelial cells of the blood-brain barrier /32/. The function of the CSF may be to provide a reservoir of extracellular fluid in addition to mechanical protection of the brain and sink for its slag products /70/. This lack of a barrier will also allow water soluble substances such as contrast media injected into the subarachnoid space to enter the extracellular space of the CNS and come into direct contact with the neurons. CNS penetration of metrizamide has also been demonstrated in animal experiments /74 - 76/ as well as in patients /77/. Experimental data indicate that the penetration is by simple diffusion /76/ and there is no difference between metrizamide and ionic contrast media /75/. The higher toxicity of ionic contrast media is thus not related to differences in the depth of penetration or the rate of diffusion. However, factors which may influence the parenchymal concentration of a contrast medium are important since a direct relationship has been demonstrated between cerebral contrast medium concentration and rate of complications following intrathecal administration of metrizamide /78/. Fenstermacher et al /76/ found a slight difference in the distribution of metrizamide in the neural tissue of rabbits compared to an extracellular marker (EDTA) and raised the suspicion of some cellular entry or binding. The result of the present study also provided some indication of metrizamide retention in neural tissue (III). In contrast to metrizamide, iohexol appears to follow a simple diffusion model (IV) and should thus result in a shorter neuron contact time provided that the two contrast media have identical absorption rates from the CSF. The present study was unable to establish the cause of metrizamide retention but intracellular localization has never been shown /74/ and it has been considered unlikely that metrizamide, even in high doses, will diffuse into brain cells /79/. The retention of metrizamide in neural tissue is therefore more likely a result of membrane binding (III).

The major problem with water soluble contrast media is their toxicity to neural tissue. The oil-based contrast media differ in this respect since they remain in the subarachnoid space and thus are not in contact directly with the neurons. The oil-based contrast media also do not cause any disturbance in the delicate balance between the extra- and intracellular ions from osmotic forces. There are, however, other problems with these contrast media, as mentioned in the introduction which make them obsolete. The conventional ionic contrast media are toxic to neural tissue, partly as a result of their osmotic effects, but mostly because of their ionic structure. The ionic contrast media can induce epileptiform activity when applied to cortical tissue /80/ which most likely occurs as a result of the free salt-forming ions and not their osmolality /81/. The effect of hyperosmolality on the CSF composition was studied by Maly et al /24/ who could demonstrate that an injection of hyperosmolar solutions of iohexol or metrizamide in the subarachnoid space of rabbits results in a relative increase of calcium and magnesium ions with respect to sodium and potassium ions. The effect was most pronounced for calcium but the median concentration was never above the pre-injection level for hyperosmolar iohexol which was the case with hyperosmolar metrizamide. Metrizamide has a similar effect also on isotonic solutions, although less pronounced. Isotonic iohexol was completely inert in this aspect and showed only signs of CSF dilution. This relative increase in calcium and magnesium ions may be the origin of the depressive effect reported from hyperosmolality /16/. Both calcium and magnesium are known to cause a depressive effect when their concentrations are increased in the CSF /21, 82/. It is probable that the effect is somewhat different in man since magnesium normally is higher in CSF than plasma /21/ in contrast to the rabbit. Both calcium and magnesium are supposed to be transported into CSF by means of active, energy dependent carrier systems /21/. The calcium level is even maintained in CSF post mor-

tem, unlike magnesium which will increase rapidly in its CSF concentration as a result of a leak from brain cells when the pumping mechanism fails /83/. The calcium content in the brain is low and it is tightly bound to intracellular structures /21/. In the present study it was also shown that hypertonicity has a marked depressive effect on glucose metabolism (II) and the depression in electrical activity seen from hypertonic iohexol by Bryan et al /16/ could eventually be the result of a shift in the magnesium gradient in neurons from an energy depleted magnesium pump. The increase in calcium concentration is harder to explain since interference with the calcium-pump in the blood-brain barrier should result in a decrease in concentration rather than an increase. If the osmotic changes were a result of an opening in the blood-brain barrier there should also be changes in the potassium concentration. The complete picture of how calcium is transported into the CSF is thus still nebulous and there may be species variations. In man the calcium concentration is uniform in the ventricles and lumbar sac, but in rabbits /84/, dogs and monkeys the lumbar levels are slightly higher /21/ and in cats slightly lower /85/. The effect of hypertonicity in CSF composition has yet to be studied in man.

In spite of the absence of free ions metrizamide has been shown to possess certain neurotoxic qualities that are not due to hyperosmolality. Bryant et al /16/ showed in electrophysiological in vitro experiments that metrizamide causes early excitatory changes in electrical activity besides a mild, late inhibitory effect from hypertonicity. Iohexol (300 mg I/ml) caused only inhibition which was more marked when the contrast medium concentration, and subsequently the osmolality of the CSF/contrast medium solution, was increased. Ionic contrast media with their higher osmolality caused more pronounced depression as well as their excitatory effects. All these effects were reversible with rinse-out within 30 minutes /16/. The origin of this excitatory effect from metrizamide has not been definitely established but the presence of 2-deoxy-D-glucose, a known competitive inhibitor of glucose metabolism /86/, in the metrizamide molecule led Bertoni et al /87/ to suggest and demonstrate a competition between glucose and metrizamide for hexokinase. Glucose is the main source of energy in the CNS which makes it extremely vulnerable to factors which interfere with glucose metabolism /88 - 89/. This vulnerability is heightened by the inability of the brain to store larger amounts of glucose as glycogen and, consequently, the brain is dependent on an almost continuous supply of glucose /90/. D-glucose in the CNS is transported intracellularly by means of a bidirectional facilitated carrier in the membrane /34/. This carrier is stereospecific and can be competitively inhibited by structural analogues such as 2-deoxy-D-glucose which does not transport L-glucose /21, 90 - 93/. In the CNS, facilitated D-glucose carriers are found in the capillary endothelium, the choroid plexus and the cell membranes of neurons and glia /34/. As a facilitated carrier, the glucose carrier is not dependent on energy and the transport will thus be downhill along a concentration gradient. The normal concentration of glucose in the blood is approximately 100 mg/dl, compared to 60 mg/dl in CSF and 20 mg/dl in the brain. The lower concentration of glucose in the brain allows it thus to act as a sink for glucose in the blood and CSF /21/.

If metrizamide does have any effect on hexokinase in vivo it either has to be transported intracellularly, or the 2-deoxy-D-glucose component, alternatively D-glucosamine /94/, has to be broken off from the metrizamide molecule since hexokinase is found only intracellularly. Although these possibilities have not been excluded, it seems unlikely either will be of any importance. Intracellular localization of metrizamide has never been proven /74, 79/, nor has a break in the metrizamide molecule following intrathecal administration been shown /95/. The present study of the effect of metrizamide on glucose metabolism (I) demonstrated that metrizamide causes a metabolic depression in vitro, shown by a reduction in carbon dioxide production. This may be due to interference with the glucose carrier in the cell membrane, either by a direct competition between glucose and the 2-deoxy-D-glucose component of metrizamide or by a distortion of the membrane carrier from an unspecific membrane binding (I). The study of the effect of iohexol on glucose metabolism (II) did not reveal any evidence of metabolic disturbance caused by the iohexol molecule. However, there was an effect

from hyperosmolality and the decrease in metabolism caused by hypertonicity in vitro was more pronounced than expected from a linear relationship (II). This osmotic effect will probably be much less significant in vivo since water rapidly diffuses towards the hyperosmotic areas. In view of the metabolic effects of metrizamide (I) and the suggestion of retention of metrizamide in neural tissue (III) compared to iohexol (IV), iohexol should be safer than metrizamide for subarachnoid examination. This assumption was also supported by the lower frequency of adverse reactions for iohexol compared to metrizamide in the present double-blind clinical trial (V).

Summary and Conclusions

The diagnostic and technical limitations of gas and oil-based myelographic contrast media stimulated the search for a non-toxic water soluble contrast medium that could be used throughout the subarachnoid space. The conventional ionic contrast media are all too toxic to be of any practical use above the lumbar region but the goal seemed finally to be realized with the introduction of metrizamide. As a non-ionic contrast medium, metrizamide lacks free ions and has a much lower osmotic effect than the ionic contrast media with identical iodine concentration. However, in spite of these improved physical characteristics, metrizamide was not completely free of neurotoxic properties and several adverse reactions have been reported in the literature. The cause of these side effects from metrizamide is not fully understood but the presence of a glucose analogue in its molecule has given rise to speculation about metabolic disturbances occurring within neural tissue.

An in vitro model using rat neural tissue was designed to examine the influence of metrizamide on neural tissue glucose metabolism. The hypothesis that interference with glucose metabolism was the result of the molecule structure was tested by comparing metrizamide to isotonic and hypertonic iohexol. The data from these studies showed that:

- . there is a depression in neural tissue metabolism from metrizamide.
- .. iohexol, which lacks a glucose analogue in its molecule, shows no evidence of interference with glucose metabolism.
- ... hypertonicity in vitro markedly depresses neural tissue metabolism.

The toxic effects will also depend on the tissue concentration and the contrast medium contact time with neurons. An animal model was designed to study neural tissue diffusion kinetics for metrizamide following lumbar myelography. This study was repeated with iohexol to evaluate if any difference in diffusion kinetics exist between the two contrast media. These two experiments showed that:

- . there was no significant difference in tissue penetration between metrizamide and iohexol. Both contrast media diffuse into the extracellular space of the lumbar cord in relation to time and concentration of the contrast medium in the CSF.
- .. both contrast media were for the most part absorbed to the blood in the lumbosacral region following lumbar myelography in rabbits.

- ... cranial transport of either contrast media was very small.
- there are indications that metrizamide is partially trapped within the cord resulting in a prolonged contact time with neurons.
- iohexol follows a simple diffusion model for tissue penetration.

To evaluate the clinical significance of these experimental findings a double-blind trial was performed to compare metrizamide and iohexol in lumbar myelography. The results of this trial showed that:

- . neither metrizamide nor iohexol caused any serious adverse reactions.
- .. the most common side effect was headache which was more prevalent following metrizamide than iohexol myelography.

The conclusions which can be drawn from the present investigation are that:

1. adverse reactions following intrathecal administration of metrizamide may be a result of disturbed neural tissue metabolism.
2. the iohexol molecule does not interfere with neural tissue metabolism.
3. in vitro experiments, to evaluate the chemotoxic properties of a contrast medium, should be performed with isotonic solutions since osmotic effects otherwise may influence the result in a way that has no bearing in vivo where water will rapidly diffuse towards hypertonic areas and reduce this effect.
4. if resorption and CSF circulation in man is similar to the experimental findings in this investigation, it is important to keep the head elevated while the contrast medium is resorbed to avoid gravitational cranial flow of contrast medium to the brain that might increase the risk for adverse reactions.
5. the difference in neural tissue diffusion kinetics for the two contrast media should favor the use of iohexol for subarachnoid examinations since potential tissue binding with metrizamide might increase the risk of adverse reactions.

References

1. Dandy WE: Röntgenography of the brain after the injection of air into the spinal canal. *Ann Surg* 1919; 70: 397 - 403
2. Jacobaeus HC: On insufflation of air into the spinal canal for diagnostic purposes in cases of tumors in the spinal canal. *Acta Med Scand* 1921; 55: 555 - 564
3. Lindgren E: Myelography with air. *Acta Psychiatr Neurol* 1939;14: 385 - 388
4. Sicard JA, Forestier JE: Methods générale d'exploration radiologique par l'huile iodée (Lipiodol). *Bull Mém Soc Méd hop Paris* 1922; 46: 463 - 468
5. Steinhausen TB, Dungan CE, Furst JB, Plati JT, Smith SW, Darling AP, Wolcott EC Jr: Iodinated organic compounds as contrast media for radiographic diagnoses. *Radiology* 1994; 43: 230 - 235
6. Mason MS, Farr J: Complications of pantopaque myelography. Case report and review. *J Neurosurg* 1962; 19: 302 - 311
7. Arnell S, Lidström F: Myelography with Skiodan (Abrodil). *Acta Radiol (Stockholm)* 1931; 12: 287 - 288
8. Campbell RL, Campbell JA, Heimbürger RF, Kalsbeck JE, Mealey J Jr: Ventriculography and myelography with absorbable radiopaque medium. *Radiology* 1964; 82: 286 - 289
9. Gonsette R, André-Balisaux G: A new hydrosoluble and resorbable contrast material for myelography and ventriculography. XII Int Congr Radiol, Tokyo, Japan, Oct 9 - 11, 1969
10. Almén T: Contrast agent design: Some aspects on the synthesis of water soluble contrast agents of low osmolality. *J Theor Biol* 1969; 24: 216 - 226
11. Gonsette RE: Cervical myelography with a new resorbable contrast medium: Amipaque. *Acta Neurol Belg* 1976; 76: 283 - 285
12. Amundsen P: Metrizamide in cervical myelography: Survey and present state. *Acta Radiol (Stockholm) Suppl No. 355*, 1977; 85 - 97
13. Dugstad G, Eldevik P: Lumbar myelography. *Acta Radiol (Stockholm) Suppl 355*, 1977; 17 - 30
14. Nickel AR, Salem JJ: Clinical experience in North America with metrizamide: Evaluation of 1850 subarachnoid examinations. *Acta Radiol (Stockholm) Suppl 355*, 1977; 409 - 416
15. Golman K, Olivecrona H, Gustafson C, Salvesen S, Almén T, Maly P: Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. *Acta Radiol (Stockholm) Suppl 362*, 1980; 83 - 86
16. Bryan RN, Centeno RS, Hershkowitz N, Poelstra RJ, Osato MS: Neurotoxicity of iohexol: A new nonionic contrast medium. *Radiology* 1982; 145: 379 - 382

17. Lossius R, Eldevik OP, Weber H, Oftedal SI, Strømme JH: First clinical trial with iohexol in myelography. *Acta Radiol Diagnosis* 1983; 24: 499 - 502
18. Batty VB: Cervical myelography using iohexol (Omnipaque), a new contrast medium. *Clin Radiol* 1984; 35: 75 - 77
19. Katzman R, Pappius HM: *Brain Electrolytes and Fluid Metabolism*. Baltimore: Williams & Wilkins, 1973
20. Lajtha A Ford DH (Eds): *Brain Barrier Systems*. Amsterdam, New York: Elsevier, 1968
21. Fishman RA: *Cerebrospinal Fluid in Diseases of the Nervous System*. Philadelphia, London, Toronto: W B Saunders Co, 1980
22. Wright EM: Transport processes in the formation of the cerebrospinal fluid. *Rev Physiol Biochem Pharmacol* 1978; 83: 1 - 34
23. Junck L, Marshall WH: Neurotoxicity of radiological contrast agents. *Ann Neurol* 1983; 13: 469 - 484
24. Maly P, Fex G: CSF cation changes following subarachnoid injection of iohexol and metrizamide in rabbits. *Acta Radiol Diagnosis* 1984; 1: 65 - 71
25. Miner LC, Reed DJ: Composition of fluid obtained from choroid plexus tissue isolated in a chamber in situ. *J Physiol (London)* 1972; 227: 127 - 139
26. Welch K: Secretion of cerebrospinal fluid by choroid plexus of the rabbit. *Am J Physiol* 1963; 205: 617 - 624
27. Cserr HF, Cooper DN, Suri PK, Patlak CS: Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am J Physiol* 1981; 240: F319 - F328
28. Rosenberg GA, Kyner WT, Estrada E: Bulk flow of brain interstitial fluid under normal and hyperosmolar conditions. *Am J Physiol* 1980; 238: F42 - F49
29. Davson H: *Physiology of the Cerebrospinal Fluid*. London: J & A Churchill, 1967
30. Welch K: The principles of physiology of the cerebrospinal fluid in relation to hydrocephalus including normal pressure hydrocephalus. In: Friedlander WJ (Ed): *Current Reviews. Advances in Neurology*. New York: Raven Press, 1975; 13: 247 - 332
31. Wood JH: Physiology, pharmacology, and dynamics of cerebrospinal fluid. In: Wood JH (Ed): *Neurobiology of Cerebrospinal Fluid*. New York: Plenum Press, 1980, pp. 1 - 16
32. Brightman MW: The intracerebral movement of proteins injected into blood and cerebrospinal fluid of mice. Lajtha A and Ford DH (Eds): *Progress in Brain Research*. Amsterdam: Elsevier, 1968; 29: 19 - 37
33. Segal MB, Pollay M: The secretion of cerebrospinal fluid. *Exp Eye Res Suppl* 1977; 25: 127 - 148
34. Bradbury MWB: *The Concept of a Blood-Brain Barrier*. Chichester NY: Wiley, 1979

35. Vates TS Jr, Bonting SL, Oppelt WW: Na-K activated adenosine triphosphatase formation of cerebrospinal fluid in cat. *Am J Physiol* 1964; 206: 1165 - 1172
36. Tschirgi RD, Frost RW, Taylor JL: Inhibition of cerebrospinal fluid formation by a carbonic anhydrase inhibitor, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (Diamox). *Proc Soc Exp Biol Med* 1954; 87: 373 - 376
37. Reed DJ: The effect of furosemide on cerebrospinal fluid flow in rabbits. *Arch Int Pharmacodyn* 1969; 178: 324 - 330
38. Buhrley LE, Reed DJ: The effect of furosemide on sodium-22 uptake into cerebrospinal fluid and brain. *Exp Brain Res* 1972; 14: 503 - 510
39. Cutler RWP, Page L, Galicich J, Watters GV: Formation and absorption of cerebrospinal fluid in man. *Brain* 1968; 91: 707 - 720
40. Hochwald GM, Wald A, Malhan C: The sink action of cerebrospinal fluid volume flow. Effect on brain water content. *Arch Neurol* 1976; 33: 339 - 344
41. Eldevik OP, Haughton VM, Sasse EA: The effect of dehydration on the elimination of aqueous contrast media from the subarachnoid space. *Invest Radiol* 1980; 15: 155 - 157
42. Oppelt WW, Maren TH, Owens ES, Rall DP: Effects of acid-base alterations on cerebrospinal fluid production. *Proc Soc Exp Biol* 1963; 114: 86 - 89
43. Harnish PP, Distefano V: Decreased CSF production by intravenous sodium diatrizoate. *Invest Radiol* 1984 (In press)
44. Lindvall M, Edvinsson L, Owman C: Sympathetic nervous control of cerebrospinal fluid production from the choroid plexus. *Science* 1978; 201: 176 - 178
45. Edvinsson L, Nielsen KC, Owman C: Cholinergic innervation of choroid plexus in rabbits and cats. *Brain Res* 1973; 63: 500 - 503
46. Haywood JR, Vogh BP: Some measurements of autonomic nervous system influence on production of cerebrospinal fluid in the cat. *J Pharmacol Exp Ther* 1979; 208: 341 - 346
47. Marder E, O'Neil M, Grossman RI, Davis KR, Taveras JM: Cholinergic actions of metrizamide. *AJNR* 1983; 4: 61 - 65
48. Dawson P: Contrast media and enzyme inhibition. I. Cholinesterase. *Brit J Radiol* 1983; 56: 653 - 656
49. Katzman R, Hussey F: A simple constant-infusion manometric test for measurement of CSF absorption. I. Rationale and method. *Neurology* 1970; 20: 534 - 544
50. Mortensen OA, Weed LH: Absorption of isotonic fluids from the subarachnoid space. *Am J Physiol* 1934; 108: 458 - 468
51. Davson H, Hollingsworth G, Segal MB: The mechanism of drainage of the cerebrospinal fluid. *Brain* 1970; 93: 665 - 678

52. Key EAH, Retzius MG: Studien in der Anatomie des Nervensystems und des Bindegewebe. Stockholm: Samsom and Wallin, 1875
53. Weed LH: Studies on cerebrospinal fluid. No. III. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. J Med Res 1914; 31: 51 - 91
54. Welch K, Friedman V: The cerebrospinal fluid valves. Brain 1960; 83: 454 - 469
55. Kido DK, Gomez DG, Pavese AM Jr, Potts DG: Human spinal arachnoid villi and granulations. Neuroradiol 1976; 11: 221 - 228
56. Tripathi RC: Ultrastructure of the arachnoid matter in relation to outflow of cerebrospinal fluid. A new concept. Lancet 1973; 2: 8 - 11
57. Lee BCP, Gomez DG, Potts DG, Pavese AM: Passage of Amipaque (metrizamide) through the arachnoid granulations. Neuroradiol 1979; 17: 185 - 190
58. Levine JE, Povlishock JT, Becker DP: The morphological correlates of primate cerebrospinal fluid absorption. Brain Res 1982; 241: 31 - 41
59. Welch K, Pollay M: Perfusion of particles through arachnoid villi of the monkey. Am J Physiol 1961; 201: 651 - 654
60. Chien S: Biophysical behaviour of red cells in suspensions. In: Surgenor D MacN (Ed): The Red Blood Cell. 2nd ed, vol II. New York: Academic Press, 1975: 1031 - 1133
61. Földi M, Csillik B, Zoltan ÖT: Lymphatic drainage of the brain. Experientia 1968; 24: 1283 - 1287
62. Bradbury MWB, Westrop RJ: Factors influencing exit of substances from cerebrospinal fluid into deep cervical lymph of the rabbit. J Physiol 1983; 339: 519 - 534
63. Bering EA Jr: Introductory remarks for conference on the cerebrospinal fluid and the extra-cellular fluid of the brain: Introductory remarks. Fed Proc 1974; 33: 2061 - 2063
64. Cathcart RS 3rd, Worthington WC Jr: Ciliary movement in the rat cerebral ventricles: Clearing action and direction of current. J Neuropath Exp Neurol 1964; 23: 609 - 618
65. DiChiro G, Hammock MK, Bleyer WA: Spinal descent of cerebrospinal fluid in man. Neurology 1976; 26: 1 - 8
66. DiChiro G: Movement of the cerebrospinal fluid in human beings. Nature (London) 1964; 204: 290 - 291
67. Golman K, Wiik I, Salvesen S: Absorption of a non-ionic contrast agent from cerebrospinal fluid to blood. Neuroradiol 1979; 18: 227 - 233
68. Henriksson L, Voigt K: Age-dependent differences of distribution and clearance patterns in normal RIHSA cisternograms. Neuroradiol 1976; 12: 103 - 107
69. Speck U, Schmidt R, Volkhardt V, Vogelsang H: The effect of position of patient on the passage of metrizamide (Amipaque), meglumine iocarmate (Dimer-

X) and ioserinate (Myelografín) into the blood after lumbar myelography. *Neuroradiol* 1978; 14: 251 - 257

70. Fenstermacher JD, Patlak CS: The exchange of material between cerebrospinal fluid and brain. In: Cserr HF, Fenstermacher JD, Fencí V (Eds): *Fluid Environment of the Brain*. New York: Academic Press, 1975: 201 - 214
71. Katzman R: Blood-Brain-CSF Barriers. In: Siegel GJ, Albers RW, Katzman R, Agranoff BW (Eds): *Neurochemistry*. Boston: Little, Brown & Co, 1976: 414 - 428
72. Brightman MW, Reese TS: Junctions between intimately apposed cell membrane in the vertebrate brain. *J Cell Biol* 1969; 40: 648 - 677
73. Oldendorf WH, Davson H: Brain extracellular space and the sink action of the cerebrospinal fluid. *Arch Neurol* 1967; 17: 196 - 205
74. Golman K: Distribution and retention of ^{125}I -labelled metrizamide after intravenous and suboccipital injection in rabbit, rat and cat. *Acta Radiol* (Stockholm) Suppl 335, 1973: 300 - 311
75. Sage MR, Wilcox J, Evill CA, Benness GT: Brain parenchyma penetration by intrathecal ionic and nonionic contrast media. *AJNR* 1982; 3: 481 - 483
76. Fenstermacher JD, Bradbury MWB, duBoulay G, Kendall BE, Radu EW: The distribution of ^{125}I -metrizamide and ^{125}I -diatrizoate between blood, brain and cerebrospinal fluid in the rabbit. *Neuroradiol* 1980; 19: 171 - 180
77. Hammer B: Short communication: The pathophysiology of intrathecally-injected contrast media. In: Felix R, Kazner E, Wegener OH (Eds): *Contrast Media in Computed Tomography*. Amsterdam, Oxford, Princeton: Excerpta Medica 1981: 52 - 57
78. Caille JM, Guibert-Trainer F, Howa JM, Billerey J, Calabet A, Piton J: Contamination encéphalique par la métrizamide après myélographie. *J Neuro-radiol* 1980; 7: 3 - 12
79. Gjedde A: The blood-brain barrier is impermeable to metrizamide. *Acta Neurol Scand* 1982; 66: 392 - 395
80. Gonsette RE: Biologic tolerance of the central nervous system to metrizamide. *Acta Radiol* (Stockholm) Suppl 335, 1973: 25 - 44
81. Hershkowitz N, Bryan RN: Neurotoxic effects of water-soluble contrast agents on rat hippocampus. Extracellular recordings. *Invest Radiol* 1982; 17: 271 - 275
82. Yamamoto C: Activation of hippocampal neurons by mossy fiber stimulation in thin brain sections in vitro. *Exp Brain Res* 1972; 14: 423 - 435
83. Naumann HN: Cerebrospinal fluid electrolytes after death. *Proc Soc Exp Biol Med* 1958; 98: 16 - 18
84. Bito LZ, Davson H: Local variations in cerebrospinal fluid composition and its relationship to the composition of the extracellular fluid of the cortex. *Exp Neurol* 1966; 14: 264 - 280
85. Cserr HF: Physiology of the choroid plexus. *Physiol Rev* 1971; 51: 273 - 311

86. Gjedde A: Modulation of substrate transport to the brain. *Acta Neurol Scand* 1983; 67: 3 - 25
87. Bertoni JM, Schwartzman RJ, van Horn G, Partin J: Asterixis and encephalopathy following metrizamide myelography: Investigations into possible mechanisms and review of the literature. *Ann Neurol* 1981; 9: 366 - 370
88. Guroff G: Carbohydrates. In: *Molecular Neurobiology*. New York and Basel: Marcel Dekker Inc, 1980: 284 - 311
89. Sokoloff L: Circulation and energy metabolism of the brain. In: Siegel GJ, Alber RW, Katzman R, Agranoff BW (Eds): *Neurochemistry* (2nd ed). Boston: Little, Brown & Co, 1976: 388 - 413
90. Crone C: General properties of the blood-brain barrier with special emphasis on glucose. In: Cserr H, Fenstermacher JD, Fencel V (Eds): *Fluid Environment of the Brain*. New York: Academic Press, 1975: 33 - 46
91. Bachelard HS: Specificity and kinetic properties of monosaccharide uptake into guinea pig cerebral cortex in vitro. *J Neurochem* 1971; 18: 213 - 222
92. Cutler RWP: Neurochemical aspects of blood-brain-cerebrospinal fluid barriers. In: Wood JH (Ed): *Neurobiology of Cerebrospinal Fluid*. New York: Plenum Press, 1980: 41 - 51
93. Sokoloff L, Reivich M, Kennedy C, des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M: The (¹⁴C) deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 1977; 28: 897 - 916
94. Brunngaber EG: Nucleotide sugars: Metabolism of glucosamine and galactosamine. In: *Neurochemistry of Aminosugars: Neurochemistry and Neuropathology of the Complex Carbohydrates*. Springfield, IL, USA: C C Thomas, 1979: 310 - 331
95. Frey K: Thin-layer chromatography of ¹²⁵I-labelled metrizamide in urine from laboratory animals. *Acta Radiol*(Stockholm), Suppl 335, 1973: 286 - 291

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Metrizamide—a Potential *In Vivo* Inhibitor of Glucose Metabolism¹

Metrizamide used as a contrast medium for examination of the subarachnoid space and its contents may produce adverse reactions, but the mechanism for these reactions is not certain. The present study demonstrated a depression of glucose metabolism in rat neural tissue by metrizamide. Sixteen paired experiments using hippocampal tissue from rats showed a depression of glucose metabolism of $24.4 \pm 4.78\%$. This depression was significant ($p < 0.001$) and was not related to a dilutional or hypertonic effect. The depression occurred at about the same time that adverse reactions are seen in patients, suggesting that the adverse reactions may be related to inhibition of glucose metabolism in the central nervous system.

Index terms: Contrast media, effects • Glucose • Metrizamide

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PRIOR TO the introduction of metrizamide, investigations of the spinal subarachnoid space were usually performed with either air or oil-based myelographic agents. Air is an undesirable contrast agent because it does not consistently demonstrate nerve roots and it can cause severe headache, nausea, and vomiting. The oil-based contrast agents are undesirable because they frequently do not permit good visualization, due to their viscosity; they may also cause a delayed inflammatory reaction of the arachnoid membrane, consequently they are usually removed at the end of the study. Metrizamide, a nonionic water-soluble contrast agent, allows good visualization of the subarachnoid space and its nerve roots due to its low viscosity (1, 2). However, metrizamide is associated with adverse reactions such as headache, nausea, vomiting, dizziness, and occasionally seizures (3-5).

The absence of a significant barrier between the cerebrospinal fluid (CSF) and the extracellular space in the central nervous system (CNS) permits metrizamide to diffuse into the extracellular space of the CNS (6-8). The concentration of metrizamide in the extracellular space is directly proportional to both the concentration of metrizamide in the CSF and the length of time it remains there. This extracellular concentration of metrizamide is important, since it may be related to the incidence of adverse reactions (4).

The CNS uses D-glucose as its main source of energy; thus, interference with glucose metabolism can damage the CNS (9, 10). Glucose metabolism may be competitively inhibited by structural analogues of D-glucose such as 2-deoxy-D-glucose (9, 11-15). Metrizamide contains a 2-deoxy-D-glucose that is suspected to be a metabolic inhibitor. Bertoni *et al.* have shown competitive inhibition of glucose metabolism *in vitro* by metrizamide using a purified, commercially available microbial hexokinase (16, 17). However, they have not shown that metrizamide penetrates the cell membrane to affect hexokinase activity in neural cells. The present study was designed to measure the inhibition of glucose metabolism by metrizamide in an attempt to explain the origin of the adverse reactions encountered when using subarachnoid metrizamide.

MATERIALS AND METHODS

The effect of metrizamide on CNS glucose metabolism was studied using rat hippocampus. The hippocampus is a folded cortical structure in the temporal lobe and has been used for electrophysiological experiments (18, 19). Eight adult Wistar rats (Charles River) were used for the study. At the beginning of each experiment the animal was decapitated with a guillotine. This allowed rapid removal of the brain in an unaltered state. The hippocampi were removed and sectioned into 250- μ m slices perpendicular to the long axis. The sectioning was performed using a TC-2 tissue sectioner (Sorvall). Following sectioning, both hippocampi were placed in an artificial CSF medium that was composed of 124 mM NaCl, 2.0 mM KCl, 1.25 mM KH_2PO_4 , 2.0 mM MgSO_4 , 26 mM NaHCO_3 , and 5 mM D-glucose. Twenty tissue sections from each hippocampus were divided into two groups of 10.

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TABLE I: Metabolic Effect of Metrizamide on Rat Hippocampus

		DPM*/µg Protein		% Deviation in Activity
Rat No.	Experiment	Control	Metrizamide	
1	1	124	86	-30
	2	144	103	-28
2	3	139	109	-21
	4	172	93	-46
3	5	93	40	-57
	6	92	66	-28
4	7	43	43	0
	8	50	45	-10
5	9	38	34	-13
	10	45	37	-18
6	11	57	33	-42
	12	31	23	-26
7	13	41	37	-10
	14	51	19	-63
8	15	35	30	-14
	16	34	39	+15
Mean SD				-24.4

* DPM = disintegrations per minute. The quencher number for counts per minute showed an efficiency of 72.7 ± 0.5%.

Each group was then placed in a test tube containing 4 ml of CSF medium. In addition to the CSF medium one of the tubes also contained 1.2 ml isotonic metrizamide (Amipaque, Winthrop Laboratories), 170 mg l/ml. The CSF medium was saturated with 95% O₂ and 5% CO₂ and the tubes were sealed and placed in a shaker bath at 37.5°C.

Four hours later the tubes were opened and 2 ml of CSF medium containing 6-¹⁴C-D-glucose (2-3 µCi [0.074-0.111 MBq] per 30 ml) was added. The tubes containing metrizamide had an additional 0.6 ml of isotonic nonlabeled metrizamide added to maintain the contrast medium concentration at 30%. The CSF medium was again saturated with 95% O₂ and 5% CO₂ before the tubes were resealed. Two hours later metabolism was stopped by injecting 1.0 ml of 2N HCl through the rubber cork, thereby also causing a release of the buffered CO₂. The metabolically released CO₂ was collected by pumping room air into the tube through one needle while forcing the CO₂ out of a second needle. The air from this second needle was transferred to a scintillation vial and bubbled through 1 ml of hyamine hydroxide (Baker Chemical Co.). The CO₂, including the metabolically released ¹⁴CO₂, was bound by the hyamine hydroxide. After 20 minutes of CO₂ collection, 10 ml of Ultrafluor Scintillation Fluid (National Diagnostic) was added to the hyamine hydroxide. To evaluate the background count rate an extra test tube was used for each experiment; this was processed in the same manner as the other tubes, except that no metrizamide or hippocampal tissue was added. The count rate for the

TABLE II: Dilution Effect of Polyethylene Glycol on Rat Hippocampal Metabolism

		DPM*/µg protein		% Deviation in Activity
Rat No.	Experiment	Control	Polyethylene Glycol	
1	1	65	197	+203
	2	147	182	+24
2	3	168	166	-1
	4	163	156	-4
3	5	56	82	+46
	6	70	100	+43
4	7	55	60	+9
	8	100	25	-75
5	9	32	43	+34
	10	31	37	+19
6	11	51	50	+2
	12	47	50	+6
7	13	30	32	+7
	14	40	40	0
8	15	44	34	-23
	16	42	34	-19
Mean SD				+16.9

* DPM = disintegrations per minute. The quencher number for counts per minute showed an efficiency of 72.7 ± 0.5%.

background sample was always less than 7.5% of the test count rates. The ¹⁴CO₂ content was measured using a Beckman 8100 automatic liquid scintillation system. At the end of the experiment, the tissue was washed in saline and the amount of tissue in each sample was determined using a spectrophotometric protein assay (Bio-Rad Lab). The metrizamide did not interfere with the assay. Metabolic activity was expressed as disintegrations per minute per µg of protein.

The addition of metrizamide caused a dilution of the CSF media. The effect of dilution was studied by replacing metrizamide with an isotonic polyethylene glycol (molecular weight = 400) solution and repeating the metabolic study with another eight rats.

RESULTS

Sixteen paired hippocampal samples were obtained from eight rats to study the effect of metrizamide on glucose metabolism (TABLE I). In all but two of the paired samples (no. 7 and no. 16) metrizamide produced a depression in metabolic activity when compared with the control. The mean depression and standard error was 24.4 ± 4.78%. This depression is significant at *p* < 0.001 (Student t-test for paired samples).

In another 16 paired samples isotonic polyethylene glycol solutions were used instead of metrizamide to study the effect of dilution (TABLE II). The results with polyethylene glycol were not as consistent as the results with metrizamide and at least 2 samples (no. 1 and no. 8) deviated markedly from the remainder. There was no

metabolic depression secondary to the dilution of the CSF medium by polyethylene glycol. Instead there appeared to be a trend toward increased metabolic activity.

The experiments were performed over several days and the CSF media, including those with ¹⁴C-labeled glucose, were prepared from concentrated stock solutions daily. The concentration of radioactively labeled glucose varied slightly on different days and thus resulted in slight variation in activity between samples. This variation in activity from day to day only allowed comparisons between individual paired experiments. However, we could still compare the percent deviation in metabolic activity between paired samples to evaluate the effect of metrizamide or polyethylene glycol on glucose metabolism.

To minimize variation in tissue metabolism in different rats, we compared the results from the same hippocampus. In spite of this there were still some variations, since the percentage of viable tissue in each separate section could not be estimated. Variation in viability probably accounts for the differing results found in two of the samples in the polyethylene glycol studies (TABLE II). There may also have been a leakage of ¹⁴CO₂.

DISCUSSION

Epileptic activity due to cellular toxicity can be induced by ionic contrast agents (19-22). Metrizamide has produced similar neurological side effects, although they occur less frequently. The mechanism for these reactions has not been elucidated. One possible cause could be interference with nerve cell metabolism that reduces the amount of energy available to sustain the function of the sodium-potassium pump. Since glucose is the main source of CNS energy, the brain is extremely vulnerable to situations that interfere with glucose metabolism and this vulnerability is increased by the inability of the brain to store large amounts of glucose as glycogen (9-11, 23).

D-glucose is transported across membranes into the cells of the CNS by means of a facilitated membrane carrier. This is a saturable, stereospecifically and bidirectionally acting carrier that can be competitively inhibited by structural analogues, for example 2-deoxy-D-glucose or D-glucosamine. L-glucose is not transported by this carrier (9, 11-15). In the CNS, facilitated D-glucose carriers are found in the capillary endothelium, the choroid plexus, and the cell membranes of neurons and glia. The transport

mechanism is a form of equilibrating system and low glucose concentration in the brain allows it to act as a sink for glucose in the blood and CSF (24).

Metrizamide is a nonionic compound that may be associated with side effects when used for myelograms. The pathogenesis for these reactions is not clear but may be related to the 2-deoxy-D-glucose part of the molecule. Metrizamide is composed of a 2-deoxy-D-glucose bound to metrizoate with an amide linkage. The 2-deoxy-D-glucose is a structural analogue of D-glucose. This compound is known to inhibit glucose metabolism in neural tissue and there is a competitive inhibition between D-glucose and 2-deoxy-D-glucose for the specific glucose membrane carrier (12). Moreover, 2-deoxy-D-glucose will competitively inhibit hexokinase *in vitro*, but this is probably without clinical significance since the hexokinase reaction is extremely rapid (12, 25). Also, 2-deoxy-D-glucose and other structural analogues of glucose can inhibit other intracellular enzymes of glucose metabolism (9, 14, 26).

The aim of this study was to discover if metrizamide produced any appreciable metabolic inhibition in neural tissue, either from interference with the membrane-carrier system for glucose or from inhibition of enzymes involved in intracellular glucose metabolism. For this purpose we used 6-¹⁴C-D-glucose, which cannot be used for CO₂ production *via* the pentose monophosphate pathway. We expected and found a decrease in ¹⁴CO₂ production but continue to be uncertain whether the inhibition occurred with the membrane-carrier system or the enzymes.

Since more serious side effects have been found when the concentration of metrizamide in the tissue was higher than expected, it was decided to use a high concentration of metrizamide, but still to remain within isotonic levels. This resulted in a significant degree of dilution of the CSF. However, in the experiment on the effect of dilution, using polyethylene glycol as a control, similar changes were not seen. If the inhibition occurs at enzymatic levels, a certain time for accumulation of 2-deoxy-D-glucose-phosphate or D-glucosamine-6-phosphate would be expected before the inhibition occurs.

This might explain why most side effects following metrizamide myelography do not occur until 4–8 hours later. Whatever the mechanism, it may be possible to avoid the side effects caused by metrizamide by replacing the 2-deoxy-D-glucose on the molecule with another compound that is not transported by the membrane carrier, for example, the levoform of 2-deoxy-glucose.

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References

- Sackett JF, Strother CM, Quagliere CE, Javid MJ, Levin AB, Duff TA. Metrizamide—CSF contrast medium. Analysis of clinical application in 215 patients. *Radiology* 1977; 123:779–782.
- Skalpe IO, Torbergsen T, Amundsen P, Presthus J. Lumbar myelography with metrizamide. *Acta Radiol [Suppl] (Stockh)* 1973; 335:367–379.
- Nielsen H. Epileptic seizures following cervical myelography. *Neuroradiology* 1975; 10:59–60.
- Paling MR, Quindlen EA, DiChiro G. Spinal seizures after metrizamide myelography in a patient with a spinal block. *AJR* 1980; 135:1091–1092.
- Skalpe IO. Adverse effects of water-soluble contrast media in myelography, cisternography and ventriculography. *Acta Radiol [suppl] (Stockh)* 1977; 355:359–370.
- Bradbury M. Introduction to the blood-brain-cerebrospinal fluid system. In: Bradbury M, ed. *The concept of a blood-brain barrier*. Chichester, New York, Brisbane, Toronto: John Wiley & Sons, 1979:1–36.
- Rall DP, Oppelt WW, Patlak CS. Extracellular space of brain as determined by diffusion of inulin from the ventricular system. *Life Sci* 1962; 1:43–48.
- Fenstermacher JD, Bradbury MWB, duBoulay G, Kendall BE, Radu EW. The distribution of ¹²⁵I-metrizamide and ¹²⁵I-diatrizoate between blood, brain and cerebrospinal fluid in the rabbit. *Neuroradiology* 1980; 19:171–180.
- Guroff G. Carbohydrates. In: Guroff G, ed. *Molecular neurobiology*. New York and Basel: Marcel Dekker, Inc., 1981:284–311.
- Sokoloff L. Circulation and energy metabolism of the brain. In: Siegel CJ, Alber RW, Katzman R, Agranoff BW. *Neurochemistry*. 2d ed. Boston: Little, Brown and Co. 1972:388–413.
- Crone C. General properties of the blood-brain barrier with special emphasis on glucose. In: Cserr H, Fenstermacher JD, Fencel V, eds. *Fluid environment of the brain*. New York-San Francisco-London: Academic Press 1975:33–46.
- Bachelard HS. Specificity and kinetic properties of monosaccharide uptake into guinea pig cerebral cortex *in vitro*. *J Neurochem* 1971; 18:213–222.
- Cutler RWP. Neurochemical aspects of blood-brain-cerebrospinal fluid barriers. In: Wood JH, ed. *Neurobiology of cerebrospinal fluid*. New York and London: Plenum Press, 1980:41–51.
- Sokoloff L, Reivich M, Kennedy C, et al. The [¹⁴C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 1977; 28:897–916.
- Fishman RA. Composition of cerebrospinal fluid. In: Fishman RA, ed. *Cerebrospinal fluid in diseases of the nervous system*. Philadelphia-London-Toronto: WB Saunders Company, 1980:208–215.
- Bertoni JM, Alexander GM, Schwartzman RJ. Metrizamide competitively inhibits hexokinase. *Ann Neurol* 1980; 8:107.
- Bertoni JM, Schwartzman RJ, Van Horn G, Partin J. Asterixis and encephalopathy following metrizamide myelography: Investigations into possible mechanisms and review of the literature. *Ann Neurol* 1981; 9:366–370.
- Andersen P, Bliss TV, Skrede KK. Unit analysis of hippocampal population spikes. *Exp Brain Res* 1971; 13:208–221.
- Yamamoto C. Intracellular study of seizure-like afterdischarges elicited in thin hippocampal sections *in vitro*. *Exp Neurol* 1972; 35:154–164.
- Gonsette RE. Biologic tolerance of the central nervous system to metrizamide. *Acta Radiol [suppl] (Stockh)* 1973; 335:25–44.
- Johnston D, Brown TH. Giant synaptic potential hypothesis for epileptiform activity. *Science* 1981; 211:294–297.
- Yamamoto C. Activation of hippocampal neurons by mossy fiber stimulation in thin brain sections *in vitro*. *Exp Brain Res* 1972; 14:423–435.
- Thompson MD, Bachelard HS. Cerebral-cortex hexokinase. Comparison of properties of solubilized mitochondrial and cytoplasmic activities. *Biochem J* 1970; 118:25–34.
- Fishman RA. Physiology of the cerebrospinal fluid. In: Fishman RA, ed. *Cerebrospinal fluid in diseases of the nervous system*. Philadelphia-London-Toronto: WB Saunders Company, 1980:19–43.
- Bachelard HS, Goldfarb PS. Adenine nucleotides and magnesium ions in relation to control of mammalian cerebral-cortex hexokinase. *Biochem J* 1969; 112:579–586.
- Brunngraber EG. Nucleotide sugars. In: Brunngraber EG. *Neurochemistry of amino sugars*. Springfield, IL: Charles C. Thomas, 1979:310–331.

THE EFFECT OF IOHEXOL ON GLUCOSE METABOLISM
COMPARED TO METRIZAMIDE

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ABSTRACT

In a previous *in vitro* study we demonstrated reduced CO₂ production in rat hippocampal tissue when metrizamide was added. This metabolic depression is believed to be a result of the 2-deoxy-D-glucose (2-DG) portion of the metrizamide molecule since 2-DG is a known competitive inhibitor of glucose metabolism. This competitive inhibition probably occurs at the cell membrane since it has never been shown that metrizamide penetrates neural cells. Further the inhibition is most likely related to competition for the membrane glucose carrier. A new non-ionic contrast medium, iohexol, does not contain a 2-DG component and if the hypothesis for the metabolic inhibition is valid we should not expect metabolic inhibition with iohexol.

This hypothesis was tested using the rat hippocampus model previously used for metrizamide. We compared iohexol to metrizamide in isotonic concentrations and also examined the effect of hypertonicity. These experiments did not demonstrate inhibition of CO₂ production with iohexol at near physiologic osmolalities, however, there was a marked depressive effect with increasing osmolality. This effect from hypertonicity is, however, probably of less importance *in vivo* where water will rapidly diffuse towards the hypertonic areas. The apparent lack of interference of the iohexol molecule on glucose metabolism should therefore make iohexol a more suitable contrast medium, for subarachnoid investigations than metrizamide.

Key words: 1/ iohexol, 2/ metrizamide, 3/ *in vitro* glucose metabolism, 4/ myelography

Examination of the subarachnoid space with contrast media (CM) is important in diagnosing lesions in the spinal canal. Unfortunately, water-soluble CM injected into the subarachnoid space will diffuse into the extracellular space (ECS) of the central nervous system (CNS) and come in direct contact with the neurons /1/. Ionic CM are unsuitable for subarachnoid investigations since they dissociate rapidly and their cations and anions interfere with neuronal function. The non-ionic water-soluble CM metrizamide seemed, at first, to be an answer to these problems. However, with increasing numbers of examinations and with larger volumes it became evident that, although metrizamide was far less toxic than the ionic CM, it was still associated with adverse effects /2 - 5/. The etiology of these adverse reactions has been widely discussed but is not yet completely understood.

Metrizamide consists of a 2-deoxy-D-glucose (2-DG) linked to a triodobenzamide derivative with an amide linkage. 2-DG itself is known to competitively inhibit glucose metabolism, competing with D-glucose at the specific glucose membrane carrier as well as with certain intracellular enzymes /6 - 8/. In an *in vitro* experiment using microbial as well as human brain hexokinase, Bertoni et al demonstrated a competitive inhibition of glucose phosphorylation by metrizamide /9, 10/. In a subsequent *in vitro* study from our laboratory, a decrease in glucose metabolism was also demonstrated in rat hippocampal tissue /11/. If this depression occurs *in vivo*, this could result in a disturbance in the energy supply (ATP) of the cell which, in turn, could affect the Na/K-pump that sustains the normal extra- and intracellular ionic relationship. The second generation non-ionic, water-soluble CM, such as iohexol, have chemical structures that differ from metrizamide (Fig. 1). If the 2-DG component of metrizamide is the cause of the depression of glucose metabolism, we should not expect the same inhibitory effect with non-ionic CM of different chemical structure. In order to test this hypothesis, we examined the *in vitro* effects of iohexol on glucose metabolism on rat hippocampal tissue and compared these to the metrizamide effects.

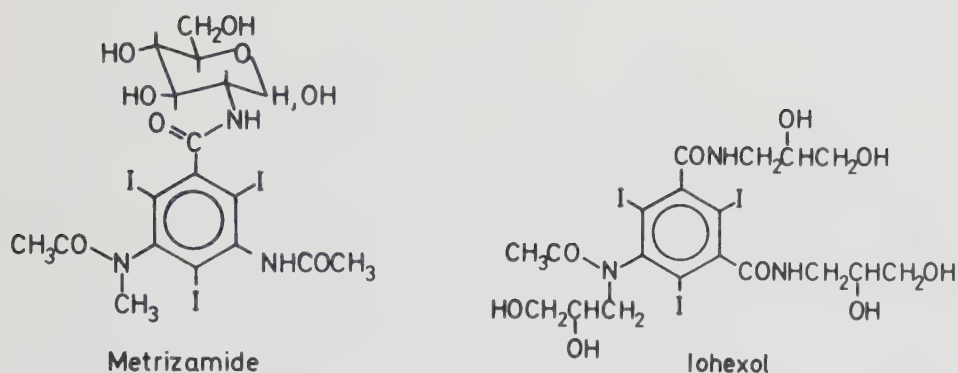


Fig. 1. Molecular structure of metrizamide and iohexol.

MATERIALS AND METHODS

Paired hippocampal tissue samples from 48 adult Wistar rats (Charles River) were used to evaluate the *in vitro* effect of iohexol on glucose metabolism in neural tissue. The animals were decapitated with a guillotine in order to allow rapid removal of the brain. The two hippocampi were removed and sectioned with a TC-2 tissue sectioner (Sorvall) into 250 μ m slices perpendicular to the long axis. The tissue slices were immediately placed in an artificial CSF-medium [11]. The sections from a single hippocampus were divided into two groups and each group was placed in a test tube containing 4 ml of CSF medium. The CSF medium in one of the paired test tubes also contained 1.2 ml of the test medium and the other 1.2 ml control medium. The CSF-medium was saturated with 95 % O_2 and 5 % CO_2 before the tubes were sealed with a rubber cork and placed in a shaker bath at 37.5°C. Four hours later the tubes were opened and 2 ml of CSF-medium containing D [U- ^{14}C] glucose (5 μ Ci [0.18 - 0.5 mBq] per 30 ml) (Amersham Int Ltd) was added. In addition, another 0.6 ml of the test and control medium was added to the respective tubes to maintain the initial concentration of these compounds. The CSF medium was once more saturated with 95 % O_2 and 5 % CO_2 before the tubes were resealed. Two hours later, metabolism was stopped by injecting 1.0 ml of 2N HCl through the rubber cork. Room air was pumped into the test tube forcing the CO_2 through a second needle to a scintillation vial containing 1 ml hyamine hydroxide. The hyamine hydroxide (Baker Chemical Co), is a strong basic solution that binds the CO_2 . Following 20 minutes of CO_2 collection, 10 ml of Ultrafluor Scintillation Fluid (National Diagnostic) was added to the hyamine hydroxide. The $^{14}CO_2$ content was measured using a Beckman 8100 automatic liquid scintillation system. The

tissue sections obtained from a single hippocampus are quite uniform. However, there were small differences in the total tissue mass between test and control groups even though the same number of tissue sections (about 12) had been used. Assuming the viability of the tissue in the two groups from the same hippocampus is identical, and that glucose metabolism is proportional to the amount of tissue we related the activity to the tissue protein content in each sample. The protein content of the tissue was determined using a spectrophotometric protein assay (Bio-Rad Lab). The activity found in the test medium was compared to its paired control and expressed as a percent deviation in activity. The results for all paired samples were statistically evaluated using Student's t-test for paired samples.

Test solutions included iohexol in concentrations of 140, 180 and 240 mg I/ml. For control we used metrizamide in concentrations of 170 and 180 mg I/ml, iohexol at 180 mg I/ml and CSF without any further agent added. The first experiment consisted of 16 rats to compare the effects of iohexol to metrizamide at 180 mg I/ml, which is a commonly used concentration of iohexol for lumbar myelography. Iohexol in this concentration is slightly hypertonic, 415 mOsm/kg water (freezing point), and produces an osmolality of about 335 mOsm/kg water for the final CSF mixture. Metrizamide at 180 mg I/ml is almost isotonic and produces a very small increase in osmolality for the final metrizamide CSF solution.

To establish what importance this osmolality difference might have had on metabolism we also compared an isotonic solution of iohexol (140 mg I/ml) to iohexol containing 180 mg I/ml and to isotonic metrizamide (170 mg I/ml). To obtain isotonic iohexol we diluted iohexol (180 mg I/ml) with 0.1 M Tris-buffer. An additional 8 rats were used for each of the studies. Finally to evaluate the importance of increased osmolality of iohexol in higher concentrations we also compared iohexol (240 mg I/ml), which has an osmolality of 500 mOsm/kg water (freezing point), to metrizamide (180 mg I/ml) and to a non-diluted, pure CSF solution. In this concentration, 240 mg I/ml, iohexol attains a final osmolality of the CSF mixture of approximately 360 mOsm/kg water. Each of these experiments was performed using another 4 rats.

In previous studies we had used an isotonic polyethylene glycol solution (MW 400) as a control to iohexol /16/ and to evaluate the effect of CSF dilution from added contrast medium /11/. Because of some doubts about its suitability as an inert control we repeated the dilution study comparing isotonic polyethylene glycol to non-diluted CSF. For this study another 8 rats were used.

RESULTS

All data from these experiments are shown in Table I. The initial experiment comparing iohexol to metrizamide, both in a concentration of 180 mg I/ml, demonstrated a 23.3 ± 11.1 % higher CO_2 production for the iohexol containing samples. This mean difference in CO_2 production is significant at a $p < 0.05$ level. In the following experiments we tried to establish if there was any impact from the slight hypertonicity of iohexol (180 mg I/ml) and compared an isotonic iohexol solution (140 mg I/ml) to the 180 mg I/ml concentration as well as to isotonic metrizamide (170 mg I/ml). The result of the first of these two experiments demonstrated a slightly higher CO_2 production ($+7.8 \pm 6.5$ %) with isotonic iohexol than with iohexol at 180 mg I/ml, however, this was not statistically significant. The CO_2 production of isotonic iohexol was significantly higher than isotonic metrizamide $+27.4 \pm 9.9$ % ($p < 0.02$). This difference was slightly greater than that seen in the initial experiment comparing the two media at 180 mg I/ml. Previously we had demonstrated a lower CO_2 production of -24.4 ± 4.7 % for isotonic metrizamide as compared to control solution /11/.

TABLE I. Experimental data

Number of paired samples	Test substance	Control	% deviation in activity	Significance
32	Iohecol 180 mg I/ml	Metrizamide 180 mg I/ml	+23.3 % $\pm$ 11.1 SEM	p < 0.05
16	Iohecol 140 mg I/ml (isotonic)	Iohecol 180 mg I/ml	+7.8 % $\pm$ 6.5 SEM	No significance
16	Iohecol 140 mg I/ml (isotonic)	Metrizamide 170 mg I/ml (isotonic)	+27.4 % $\pm$ 9.9 SEM	p < 0.02
8	Iohecol 240 mg I/ml	Metrizamide 180 mg I/ml	-17.8 % $\pm$ 7.6 SEM	p < 0.05
8	Iohecol 240 mg I/ml	No additive	-61.2 % $\pm$ 4.3 SEM	p < 0.001
16	Polyethylene glycol (isotonic)	No additive	+16.1 % $\pm$ 15.4 SEM	No significance

The results from these experiments thus far have not revealed any evidence of interference on neural tissue metabolism from iohexol. Moreover, a slightly hypertonic iohexol solution seems only to result in a mild depression of metabolism.

The final two experiments were performed to evaluate if higher concentrations of iohexol might have any negative impact on metabolism from its stronger osmotic effect. Iohexol at 240 mg I/ml produced a lower CO₂ production than did metrizamide at 180 mg I/ml. The difference of 17.8 ± 7.6 % was significant ($p < 0.05$). When iohexol in the same concentration (240 mg I/ml) was compared to a pure CSF control the CO₂ production was 61.2 ± 4.3 % lower in the iohexol samples. This is significant at a $p < 0.001$ level. Thus, there seems to be a marked impact from hypertonicity in vitro when the osmotic effect is raised above a certain level. The impact from CM osmolality is probably less marked in vivo since water will rapidly diffuse towards the hypertonic areas.

The repeated dilution study using isotonic polyethylene glycol demonstrated a 16.1 ± 15.4 % higher metabolism in the polyethylene glycol samples as compared to the non-diluted CSF control. This was not statistically significant.

DISCUSSION

The introduction of the water-soluble, non-ionic CM metrizamide as a myelographic agent has improved the diagnostic ability and quality of subarachnoid investigations. Undesirable side-effects are, however, still common with metrizamide /2 - 5/. Neurophysiological experiments have demonstrated a mild excitatory effect on field potentials in the in vitro rat hippocampus from metrizamide as well as a mild reduction of the inhibitory post synaptic potential (IPSP) /12/. Clinically, EEG changes /13/ and prolongation of brain stem evoked potentials /14/ have been demonstrated following lumbar myelography. The etiology of these metrizamide reactions has not been definitely established but our earlier in vitro study using rat hippocampal tissue indicated, by means of reduced CO₂ production, that metrizamide depressed glucose metabolism /11/, which may explain neuronal dysfunction in vivo. We hypothesized that this depression results from competitive inhibition of glucose by the 2-deoxy-D-glucose component of metrizamide, probably at the specific glucose membrane carrier, but interference with certain intracellular enzymes could not be excluded.

The second generation non-ionic, water-soluble CM, now being introduced, appear to be less neurotoxic and thus more suitable as myelographic agents. Their chemical structures differ from metrizamide in that they do not have a 2-DG component. If free or bound to metrizamide, 2-DG produces this metabolic depression, then the new non-ionic CM should not exert this effect. Neurophysiological studies of iohexol in vitro also differ since the initial excitatory changes in electrical activity seen with metrizamide are not found with iohexol. However, both media produce a late, mild inhibition in electrical activity /15/. Previously we had evaluated the effect of dilution of CSF with metrizamide on CO₂ production using isotonic polyethylene glycol /11/. This dilution study was repeated with a fresh batch of isotonic polyethylene glycol. The result, $+16.1 \pm 15.4$ % higher metabolism for polyethylene glycol samples, is identical to that previously reported /11/. However, the CO₂ production was not significantly higher because of the large variation in individual samples. This large variability creates questions about the use of polyethylene glycol as a suitable, inert control agent. We therefore performed the iohexol experiments using direct comparison to metrizamide. The result of these experiments between iohexol and metrizamide in concentrations of 180 mg I/ml as well as in iso-

tonic concentrations did not reveal any evidence for metabolic depression from iohexol. We also compared slightly hypertonic iohexol (180 mg I/ml) to an isotonic iohexol solution. This resulted in a slightly higher CO₂ production, 7.8 %, in the samples containing isotonic iohexol but this difference was not statistically significant. Because we had observed a marked difference between iohexol (180 mg I/ml) and a hypertonic polyethylene glycol solution, a more hypertonic iohexol solution (240 mg I/ml), 500 mOsm/kg water, was compared to metrizamide (180 mg I/ml) as well as to a non-diluted, pure CSF solution. Iohexol in this concentration showed a marked reduction in CO₂ production. Thus there are clear indications that the osmolality of a CM may have a strong influence on neural metabolism in vitro.

Electrophysiologic experiments in vitro have shown a mild depressive effect from iohexol (300 mg I/ml) /15/. This effect was more marked when the amount of iohexol was increased, thus increasing the osmolality of the final solution. The same was true for the other contrast media tested. This finding could be a result of direct neurotoxicity but an osmotic effect cannot be ruled out although the osmolality of the iohexol solution was never higher than about 340 mOsm/kg water. In vivo changes in osmolality in CSF from injected CM are probably even less pronounced since water movement into CSF is believed to be a result of local osmotic forces. It thus seems likely that the osmolality of the CM would be of less importance in vivo and thus toxicity studies in vitro should probably be performed using isotonic solutions.

Metrizamide, as the first non-ionic CM, has been proven superior to the ionic CM for subarachnoid investigation and initial reports clearly suggest that iohexol will provide further improvements /17/. The results of our study fail to show any decrease in neural tissue metabolism caused by isotonic iohexol. These results thus further confirm the impression that iohexol is a more suitable CM for subarachnoid investigations than metrizamide.

REFERENCES

1. Winkler SS and Sackett JF: Explanation of metrizamide brain penetration: A review. JCAT 1980; 4 (2): 191 - 193
2. Skälpe IO: Adverse effects of water-soluble contrast media in myelography, cisternography and ventriculography. Acta Radiol (Suppl) 1977: 355: 359 - 370
3. Vincent FM and Zimmerman JE: Metrizamide encephalopathy. Ann Neurol 1980; 7 (5): 494
4. Russell D, Anke IM, Nyberg-Hansen R, Slettnes O, Sortland O and Veger T: Complex partial status epilepticus following myelography with metrizamide. Ann Neurol 1980; 8 (3): 325 - 327
5. Paling MR, Quindlen EA and diChiro G: Spinal seizures after metrizamide myelography in a patient with a spinal block. AJR 1980; 135: 1091 - 1092
6. Sokoloff L: Circulation and energy metabolism of the brain. In: Siegel GJ, Alber RW, Katzman R, Agranoff BW: Neurochemistry, 2nd ed. Boston: Little, Brown & Co. 1972: 388 - 413
7. Bachelard HS: Specificity and kinetic properties of monosaccharide uptake into guinea pig cerebral cortex in vitro. J Neurochem 1971; 18: 213 - 222
8. Sokoloff L, Reivich M, Kennedy C, des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O and Shinohara M: The ^{14}C deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 1977; 28: 897 - 916
9. Bertoni JM, Schwartzman RJ, van Horn G and Partin J: Asterixis and encephalopathy following metrizamide myelography: Investigations into possible mechanisms and review of the literature. Ann Neurol 1981; 9: 366 - 370
10. Bertoni JM: Metrizamide inhibits human brain hexokinase. Neurology (NY) 1982; 32: 884 - 887
11. Ekholm SE, Reece K, Coleman JR, Kido DK and Fischer HW: Metrizamide - a potential in vivo inhibitor of glucose metabolism. Radiology 1983; 147: 119 - 121
12. Hershkowitz N and Bryan RN: Neurotoxic effects of water-soluble contrast agents on rat hippocampus - extracellular recordings. Invest Radiol 1982; 17: 271 - 275
13. Ropper AH, Chiappa KH and Young RR: The effect of metrizamide on the electroencephalogram: A prospective study in 61 patients. Ann Neurol 1979; 6 (3): 222 - 226
14. Brekkan A, Sigurjonson K, Hanneson B, Björnsson O, Snorrason E and Valdimarsson E: The penetration of metrizamide into the CNS after routine lumbar myelography as shown by CT scan and its effect on auditory brain-stem transmission time. Europ J Radiol 1983/84 (In press)

15. Bryan RN, Centeno RS, Hershkowitz N, Poelstra RJ and Osato MS: Neurotoxicity of iohexol: A new nonionic contrast medium. Radiology 1982: 145: 379 - 382
16. Ekholm SE, Reece K, Foley M, Kido DK and Morris TW: The effect of iohexol on glucose metabolism compared to metrizamide. Invest Radiol 1983: 18 (4): 514
17. Iohexol. A non-ionic contrast medium. Pharmacology and toxicology. Acta Radiol Diagnosis Suppl 362 (Ed: Lindgren E), 1980: 1 - 134

METRIZAMIDE LUMBAR MYELOGRAPHY IN RABBITS -

A STUDY OF CONTRAST MEDIA

PENETRATION AND RESORPTION

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ABSTRACT

Neurotoxicity from subarachnoid contrast media is probably related to their specific pharmacologic effects and to their penetration into the central nervous system. The lack of a barrier between the cerebrospinal fluid and the extracellular fluid of the brain and cord allows water-soluble contrast media to diffuse into the neural tissue. The aim of this study was to develop a method that allows one to quantify the neural tissue penetration for a given contrast medium in relation to the cerebrospinal fluid concentration and contact time and apply this to the use of metrizamide. The result shows a good correlation between iodine concentration in the lumbar cerebrospinal fluid and that in the lumbar cord suggestive of a simple diffusion. When time of sacrifice (contact time) is added as a covariant there is also some indication of retention of metrizamide in the neural tissue. The study also demonstrates that the resorption of the contrast medium in the rabbit in this experimental model is mainly in the lumbo-sacral region.

INTRODUCTION

The introduction of metrizamide, the first non-ionic, water-soluble contrast medium, improved our diagnostic ability to visualize the subarachnoid space. The use of metrizamide has, however, been accompanied with serious adverse reactions, whose origin is not yet completely understood /6/. It was originally assumed that metrizamide remained within the subarachnoid space until it was resorbed into the vascular system along with the cerebrospinal fluid (CSF). The interface between the CSF and central nervous system (CNS) is apparently lacking a barrier like the blood-brain barrier in most areas /9/. Water-soluble substances in the CSF, like many of the radiographic contrast media, can thus pass through the pia mater and the ependyma overlying the neural tissue and come in direct contact with the neurons. Penetration of metrizamide into the CNS following myelography has also been demonstrated with CT in animal experiments /2/ as well as clinically /5/. Fenstermacher et coll /4/ after intraventricular perfusion of contrast media in rabbits, showed a time correlated penetration into the cerebral tissue. They also found a slight difference in tissue distribution of metrizamide compared to an extracellular marker (EDTA) that raised the suspicion of some cellular entry or binding of metrizamide.

Lumbar myelography provides a high concentration of subarachnoid contrast medium in a localized area which may result in marked penetration of the contrast medium into the spinal cord. With CT such uptake of contrast medium can be demonstrated but will not allow an accurate evaluation of the eventual difference in tissue penetration between various contrast media. The aim of this study was to develop a method that allows us to quantify the neural tissue concentration of a contrast medium in relation to the CSF concentration and contact time, and to apply this to examine the distribution of metrizamide. Such a method should make it possible to compare different contrast media and to discover variations in tissue diffusion which may be of importance in selecting a contrast medium for intrathecal use.

MATERIALS AND METHODS

Twenty-nine New Zealand white rabbits weighing between 2.5 and 4.8 kg were used in this study. These 29 animals were divided into 5 groups. Four of the experiment groups received 0.5 ml of metrizamide (180 mg I/ml) injected in-

to their lumbar subarachnoid space. Groups 1 and 2 (7 animals in each) were sacrificed at one and 2 hours, respectively. Groups 3 and 4 (6 rabbits in each) were sacrificed at 4 and 8 hours, respectively. The fifth group (3 animals) received 0.5 ml of metrizamide solvent instead of the actual contrast medium.

The rabbits were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and repeated doses of the same drug were used when needed. Supplementary local anesthesia (1 % lidocaine) was given for the surgical procedures which were begun by exposing and catheterizing one of the femoral arteries with a 4 F catheter. The tip of the catheter was positioned in the abdominal aorta and used to monitor blood pressure, heart rate and blood gases. The intraarterial catheter was flushed with an isotonic solution containing 140 mM glucose, 70 mM Na⁺, 45 mM Cl⁻ and 25 mM CH₃COO⁻. In addition, the body temperature was monitored with a rectal probe. The animals were then placed prone on a restraining board, the lumbar and sacral regions were shaved and a midline incision was made over the L3 - S1 spinal processes. Bleeding was controlled by cauterization. The muscles and fascia were separated from the spinous processes by blunt dissection to minimize bleeding. The spinous processes between L5 - L7 were excised and the lamina of L6 - L7 were removed with a pair of rongeurs. Bleeding from the bone was controlled with bone wax. The ligamentum flavum and the epidural fat were removed and the dura was punctured with a 22 gauge needle. A soft guidewire was inserted caudally along the spinal cord and a short PE 10 polyethylene catheter was inserted over the guidewire. The tip of the catheter was placed at the L7 - S1 interspace. The proper placement of the catheter within the subarachnoid space was tested by allowing a few droplets of CSF to drip out the end of the catheter. The rostral end of the restraining board was then placed horizontally to minimize hydrostatic pressure while the catheter entrance through the dura was sealed with cyanoacrylate. When the cyanoacrylate had dried and the rostral end of the restraining board was elevated 30° and kept in this position throughout the experiment to avoid gravitational cranial flow.

A total of 0.5 ml of metrizamide (180 mg I/ml) or metrizamide solvent (control) was injected over a 3 minute period while arterial pressure, temperature and blood gases were monitored. The localization of the contrast medium was verified radiographically. At the end of the experiment the animals were sacrificed with an overdose of pentobarbital and a 0.2 ml sample of CSF was taken from the suboccipital area and another 0.2 ml from the lumbar area to determine the iodine content in the CSF. The spinal cord was exposed and 1 gram of tissue was taken from lower lumbar, mid-thoracic and upper cervical cord. The covering membranes were peeled from the cord and then the cord was rinsed very briefly in saline to eliminate contrast medium from its surface.

The iodine concentrations in the spinal cord samples were analyzed in an X-ray excitation fluorescence spectrophotometer (XES) /7/. Our system utilizes an isotope (²⁴¹Am) to excite the electrons in the sample. As the electrons return to their original states they fluoresce at energy levels characteristic for each element. A Ge-detector (Canberra), cooled with liquid nitrogen, is used to detect these emissions. A multichannel pulse height analyzer (Canberra) counts the number of pulses received at discreet energy levels. The amount of an element present, such as iodine, is then directly related to the number of pulses observed at its characteristic fluorescence energy. The XES system we utilize is able to measure iodine concentrations as low as 0.01 mg/ml. The iodine content in each sample was finally expressed as mg I/g of tissue.

RESULTS

In one of the 2 hour animals there was a radiographically demonstrated compression of the dural sac in the lumbar region with incomplete filling of the subarachnoid space at this level (Fig. 1 "a"). As a result of this most of the contrast medium was located above the level of the lumbar cord and this animal has been excluded from further evaluation of the cranial contrast medium circulation. The other animals showed an even filling of the lower portion of the dural sac with the upper demarcation of the contrast medium generally at the level of the L1 - L2 vertebrae. The control of blood gases, blood pressure, heart rate and body temperature were all normal and constant during the experiment except for a mild metabolic acidosis in 3 animals. The control animals did not differ in any respect from the metrizamide animals. One of the 3 control rabbits also developed a mild metabolic acidosis. In 3 animals lumbar CSF samples were not obtained at the end of the experiment.

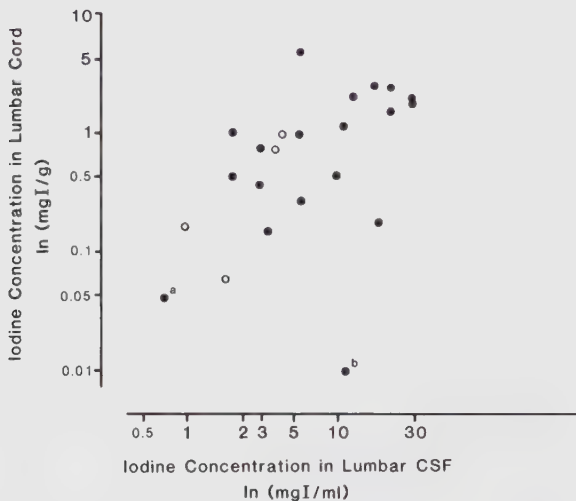


Fig. 1. The iodine concentration in the lumbar cord is plotted versus the concentration in the lumbar cerebrospinal fluid. Data points with open circles or marked "a" or "b" are discussed in the text.

The graph of the contrast medium concentration in lumbar CSF versus lumbar cord shows that all points but one are relatively close to an imagined regression line (Fig. 1). The sample that deviates most has a proportionately low contrast medium concentration in the lumbar cord in relation to the lumbar CSF concentration (Fig. 1 "b"). Before sampling lumbar CSF for evaluation of the contrast medium concentration we allowed a few droplets of CSF to drip out the end of the catheter. The disparate sample may be the result of some backflow of CSF immediately following contrast medium injection, as a result of incomplete sealing of the catheter, giving rise to a false, high CSF concentration. Due to the obvious disparity in results this animal has been excluded from further discussion. The

apparent linear relationship between the other samples seems to be consistent with a simple diffusion model (Fig. 1). If, however, the time of sacrifice is added as a covariant each group appears to have a separate regression line, the one hour group being most widely separated from the 8 hour group (Fig. 2), indicating some retention of metrizamide in the tissue. The difference in interception between the 4 regression lines was tested with the Bonferroni method of multiple comparisons at an overall level of significance of 0.05. The 8 and one hour lines differed significantly while the others were not significant.

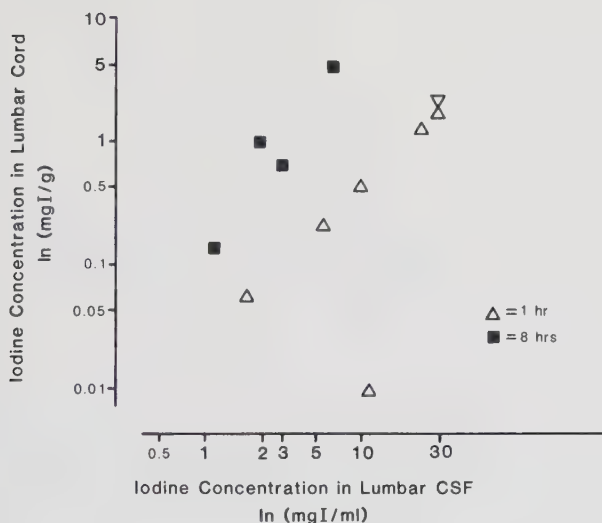


Fig. 2. The iodine concentration in the lumbar cord versus the lumbar cerebrospinal fluid for the one and 8 hour animals. A significant difference in the concentration relationship was observed between these 2 groups of animals.

The post-injection radiographs indicated possible minor damage to the arachnoid membranes in 4 animals with a small amount of contrast medium localized subdurally. This initial extra-arachnoidal contrast medium deposition should not interfere with the relationship between the lumbar cord and lumbar CSF contrast medium concentration since it is related to their values at the time of sacrifice. These 4 animals are shown as open circles in Fig. 1. However, it is important to know the exact amount of contrast medium injected into the subarachnoid space for the evaluation of the mean tissue or CSF concentration over time. These 4 animals have thus been excluded from further evaluation (Tables 1 - 2, Figs. 3 - 5). In spite of this there is a large spread in the concentration of contrast medium within each group of animals as seen in the CSF samples (Figs. 3 - 4), and the neural tissue (Fig. 5). The highest concentration found in the lumbar cord was 6.3 mg I/g of tissue in one of the 8 hour animals, which is 7 % of the total amount of iodine injected. This animal may have had some resorptive block in the spinal region since there is a marked cranial flow of contrast medium with high concentrations at all levels including the suboccipital CSF. The large concentration difference demonstrates the interanimal variation in CSF/contrast medium resorption to the blood. Although the gross mean contrast

TABLE I. Mean iodine concentration (mg I/g tissue) in the spinal cord at one, 2, 4, and 8 hours after administration of metrizamide into CSF.

Sample	1 hour	2 hours	4 hours	8 hours
Lumbar cord	1.45 [±] 0.44 SEM	0.83 [±] 0.33 SEM	1.36 [±] 0.59 SEM	1.91 [±] 1.12 SEM
Thoracic cord	0.01 [±] 0.01 SEM	0.06 [±] 0.02 SEM	0.28 [±] 0.16 SEM	1.28 [±] 1.01 SEM
Cervical cord	0	0.02 [±] 0.01 SEM	0.04 [±] 0.02 SEM	0.35 [±] 0.31 SEM
Number animals	5	5	5	5

TABLE II. Differences (in percent) in concentration of metrizamide between the three parts of the spinal cord. All intra-animal percent differences are significant ($p < 0.01$) using Student's t-test.

Time	D i f f e r e n c e s , %		Number of animals
	$(\frac{Th}{L} - 1) 100$	$(\frac{Cerv}{L} - 1) 100$	
1 hour	-99% [±] 2 SEM	-99% [±] 0.4 SEM	5
2 hours	-94% [±] 2 SEM	-97% [±] 1.9 SEM	5
4 hours	-86% [±] 4 SEM	-96% [±] 1.9 SEM	5
8 hours	-62% [±] 15 SEM	-90% [±] 4.1 SEM	5

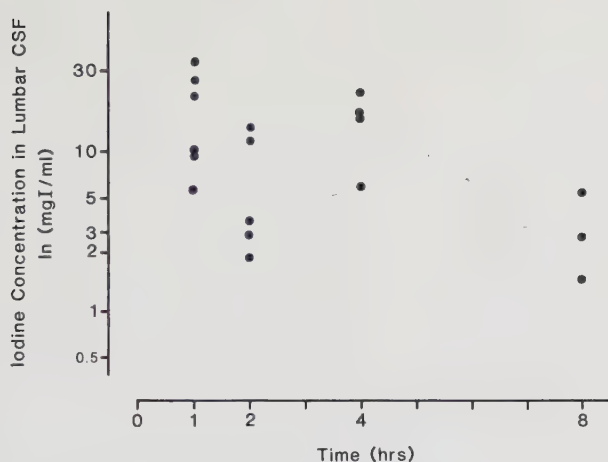


Fig. 3. The iodine concentration in lumbar cerebrospinal fluid versus time. The data show large inter-animal variability.

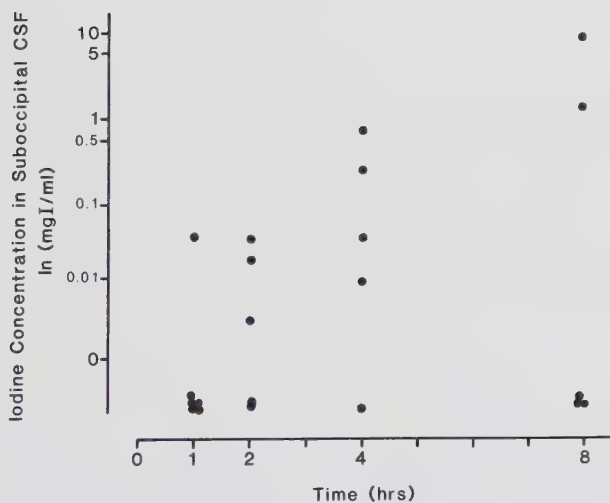


Fig. 4. The iodine concentration in suboccipital cerebrospinal fluid versus time. The suboccipital cerebrospinal fluid concentrations were generally much lower than the lumbar concentrations.

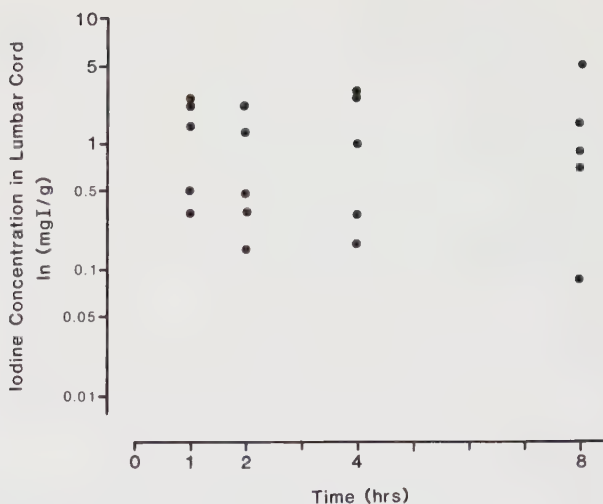


Fig. 5. The iodine concentration in lumbar cord versus time. The data show a large inter-animal variability similar to that for the cerebrospinal fluid concentrations.

medium concentration data for the cord over time indicate some cranial clearance of CSF contrast medium, these data do not show any statistical significance as a result of the large variation between individual animals (Table I). The contrast medium concentration is always lower in the thoracic and cervical cord than the lumbar cord in each individual animal. The percent difference in contrast medium concentration between the lumbar cord and the thoracic or cervical cord for each animal is statistically significant ($p < 0.01$) for each group (Table II). The decrease in percentage difference over time also demonstrates some cranial flow in this experimental design although this flow is rather small as seen from the data in Table II, as well as in the changes in CSF concentration over time (Figs. 3, 4).

DISCUSSION

The apparent absence of a barrier between CSF and CNS allows water-soluble myelographic contrast media to diffuse into the extracellular space of the CNS and come in direct contact with the neural cells. The adverse neurologic effects of a contrast medium will be related thus to both its specific pharmacologic effects and the neural tissue concentration achieved. Neural tissue penetration is a diffusion process and thus related to the molecular structure of the contrast medium, the concentration gradient and the contact time. The water-soluble contrast media are cleared from the subarachnoid space by resorption into the bloodstream along with the CSF and consequently the most important factor in neural tissue penetration for a specific contrast medium is the turn over rate for CSF. However, if there is a block of resorption from the spinal arachnoid granulations, for example as a result of chronic arachnoiditis, the contrast medium has to be transported cranially. This

transport process is apparently rather slow when the animal is immobilized and the rostral end elevated. Moreover, a spinal resorption block will result in prolonged spinal cord contact time and increased tissue concentration. The resultant increase in intracranial contrast medium concentration will further increase the risk of serious adverse neurologic effects.

Since the molecular structure of solutes may have an impact on diffusion, intracellular uptake or membrane binding, it is important to evaluate eventual differences between the various contrast media in CNS uptake. The aim of this study was to develop a method that allowed us to compare contrast media uptake by direct measurement of the spinal cord and CSF concentrations. A lumbar laminectomy was necessary to control the dose and localization of the contrast medium in the lumbar subarachnoid space. The spinal cord in rabbits extends the length of the lumbar canal and the laminectomy allowed us to control the position of the catheter and to avoid damage to the cord. External leakage of contrast medium and CSF was stopped by gluing the catheter to the dura and the tilted position of the animal hindered gravitational cranial flow. Moreover, to avoid a pressure related increase in cranial flow of the contrast medium it was injected slowly over a 3 minute period /11/. The demonstrated increase in mean concentration of contrast medium in the thoracic and cervical cord as well as in the suboccipital CSF over time is suggestive of a cranial circulation (Table I). There is also statistical proof of some flow when intra-animal differences in tissue concentration are used (Table II) although the amount that reaches the cervical cord and suboccipital CSF in most cases is very small. The main resorption of the contrast medium thus appears to take place in the lumbo-sacral region.

There is normally a large inter-animal variation in CSF production rate but it has also been shown that changes in serum osmolality /1,10/ and pH /8/ will further affect the bulk of CSF. We did not monitor serum osmolality in this study but all animals had free access to water and it seems unlikely that serum osmolality changed during this study since fluid losses were compensated for with an isotonic solution of glucose, sodium chloride and sodium acetate. The mild acidosis that developed in a few animals probably did not significantly affect the CSF production /8/. The large variation in CSF and tissue concentration and especially the lower mean concentration of contrast medium seen in the 2 hour animals, compared to the 4 and 8 hour animals, is thus probably a reflection of the inter-animal variation in CSF production. Differences in weight between animals did not seem to contribute to this variation. It is, of course, not possible to exclude some changes produced by the operative procedure, but the results (Table I) clearly demonstrate that it is not possible to ignore the actual CSF concentration at the time of sacrifice and to look at the neural tissue concentration alone.

The radiographs taken immediately following the injection of metrizamide raised the suspicion of minor damage to the arachnoid membrane in 4 animals resulting in a subdural localization of a small amount of the contrast medium. Consequently it is not possible to verify the exact amount of contrast medium initially located in the CSF in these 4 animals. It should be possible to use this animal data in the evaluation of the relationship between the actual CSF and lumbar cord concentration at the time of sacrifice since this correlation should not be affected by a somewhat lower initial CSF concentration. This relationship at first appears to be consistent with a simple diffusion model (Fig. 1), however, when the time of sacrifice is added as a covariate there are indications of some retention of metrizamide in the cord (Fig. 2). We did not attempt to actually localize the contrast medium in this study but we cannot exclude that the indicated retention of metrizamide is a result of intracellular uptake, although this has never been demonstrated. The chemical structure of metrizamide may, however, suggest an extracellular binding, e.g. a binding of the 2-deoxy-D-glucose part of metrizamide to the specific glucose membrane carrier. Such a binding could also explain the reduction in CO₂ production observed in vitro in rat hippocampal tissue when metrizamide was added /3/.

REFERENCES

1. DiMALTIO J, HOCHWALD G.M., MALHAN C. and WALD A.: Effects of changes in serum osmolarity on bulk flow of fluid into cerebral ventricles and on brain water content. *Pflügers Arch* 359 (1975), 253 - 264
2. DUBOIS P.F., DRAYER B.P., SAGE M., OSBORNE D. and HEINZ E.R.: Intramedullary penetrance of metrizamide in the dog spinal cord. *AJNR* 2 (1981), 313 - 317
3. EKHOLM S.E., REECE K., COLEMAN J.R., KIDO D.K. and FISCHER H.W.: Metrizamide - A potential *in vivo* inhibitor of glucose metabolism. *Radiology* 147 (1983), 119 - 121
4. FENSTERMACHER J.O., BRADBURY M.W.B., DuBOULAY G., KENDALL B.E. and RADER E.W.: The distribution of ^{125}I -metrizamide and ^{125}I -diatrizoate between blood, brain and cerebrospinal fluid in rabbit. *Neuroradiology* 19 (1980), 171 - 180
5. HAMMER B.: The pathophysiology of intrathecally-injected contrast media. Short communication in: Felix R., Kazner E. and Wegener O.H. (Eds): *Contrast Media in Computed Tomography*. Amsterdam - Oxford - Princeton, Excerpta Medica (1981), 52 - 57
6. JUNCK L. and MARSHALL W.H.: Neurotoxicity of radiological contrast agents. *Ann Neurol* 13 (1983), 469 - 484
7. MOSS A.A., KAUFMAN L. and NELSON J.A.: Fluorescent excitation analysis: A simplified method of iodine determination *in vitro*. *Invest Radiol* 7 (1972), 335 - 338
8. OPPELT W.W., MAREN T.H., OWENS E.S. and RALL D.P.: Effects of acid-base alterations on cerebrospinal fluid production. *Proc Soc Exp Biol (NY)*, 114 (1963), 86 - 89
9. SAGE M.R.: Kinetics of water-soluble contrast media in the central nervous system. *AJR* 141 (1983), 815 - 824
10. SAHAR A. and TSIPSTEIN E.: Effects of mannitol and furosemide on the rate of formation of cerebrospinal fluid. *Exper Neurol* 60 (1978), 584 - 591
11. SUGIURA K., OHWAKI K., YABE Y. and KONDO S.: Decrease in cerebrospinal fluid pressure following the pressure injection technique. *Acta Radiol Diagnos-*
is 13 (1972), 599 - 611

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NEURAL TISSUE UPTAKE AND CLEARANCE OF
IOHEXOL IN RABBIT MYELOGRAPHY

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Research Laboratory

ABSTRACT

The diffusion of water-soluble contrast media into the extracellular space of the central nervous system following subarachnoid investigation has previously been shown. As a result of this, water-soluble contrast medium will come in direct contact with the neurons and may interfere with their normal function. The toxic effects would thus be a result both of the molecular properties of the contrast medium as well as the local tissue concentration. A previous study by our group described the neural tissue uptake and clearance of metrizamide in rabbits following lumbar myelography. This study indicated some retention of metrizamide in the spinal cord probably as a result of binding of the contrast medium to the cell membrane. The mechanism for this has not yet been shown although it may relate to the binding of metrizamide via its 2-deoxy-D-glucose (2-DG) portion and the specific glucose membrane carrier.

The present study was performed to evaluate the diffusion kinetics of a new non-ionic contrast medium. With iohexol, which lacks a 2-DG component in its molecule a direct relationship between the neural tissue and CSF concentration was found which seems to follow a simple diffusion model. Since iohexol shows no sign of entrapment in the tissue, the contact time for neurons will be shorter than that seen with metrizamide assuming that their rate of drainage from the CSF is identical.

INTRODUCTION

The maintenance of the delicate ionic balance in the central nervous system (CNS) is dependent upon a functioning blood-brain barrier and the regulatory mechanisms for cerebrospinal fluid (CSF) production. The lack of a barrier between the CSF and the extracellular space (ECS) of the CNS results in a free exchange of solutes between these two compartments. Consequently water-soluble compounds like contrast media injected into the subarachnoid space will diffuse into the ECS of the CNS and come into direct contact with the neurons. Fenstermacher et coll /8/ showed the uptake of water-soluble contrast media in brain tissue of rabbits to be a result of simple diffusion and thus related to molecular configuration, contrast medium concentration in the CSF and its contact time. Neural tissue penetration of the non-ionic contrast medium metrizamide has also been shown using CT measurements in the spinal cord of dogs /14/ and patients /11/. The degree and type of adverse effects should thus be related to the specific pharmacologic effects of a contrast medium, and to the local concentration of the contrast medium in CSF and its contact time. The uptake profile for metrizamide, however, indicated a larger distribution space than expected /8/ which could be the result of intracellular transport or extracellular binding.

If intracellular transport of metrizamide does occur, it might result in depression of glucose metabolism by competitive inhibition of intracellular enzymes for glycogenolysis /3/ which could explain some of the side effects seen clinically /1/. However, Gjedde recently demonstrated that the blood-brain barrier is impermeable to metrizamide and it seems unlikely that metrizamide would attain intracellular concentrations sufficient to inhibit hexokinase in the brain /9/. Golman was unable to demonstrate intracellular uptake of metrizamide in an earlier study /10/. If intracellular uptake of metrizamide occurs in neural tissue it must be in very small amounts and it is doubtful that this concentration is sufficient to have any impact on glucose metabolism. The depression of glucose metabolism that we demonstrated in neural tissue from rat brain may be the result of extracellular binding of metrizamide to the specific glucose membrane carrier /5/.

In a study of metrizamide uptake in the spinal cord in rabbits following myelography our group also found a prolonged and higher tissue concentration of the contrast medium than was expected from the kinetics of simple diffusion /6/. In the present study we repeated these experiments using iohexol, one of the second generation, non-ionic, water-soluble contrast media that appears less neurotoxic than metrizamide. A study of iohexol's effects on glucose metabolism did not demonstrate any depression in rat neural tissue like that seen with metrizamide /7/. Although the chemical structure of these two compounds differ (Fig. 1) their molecular weights are approximately the same (821 for iohexol and 789 for metrizamide) and therefore their diffusion kinetics and distribution should be comparable.

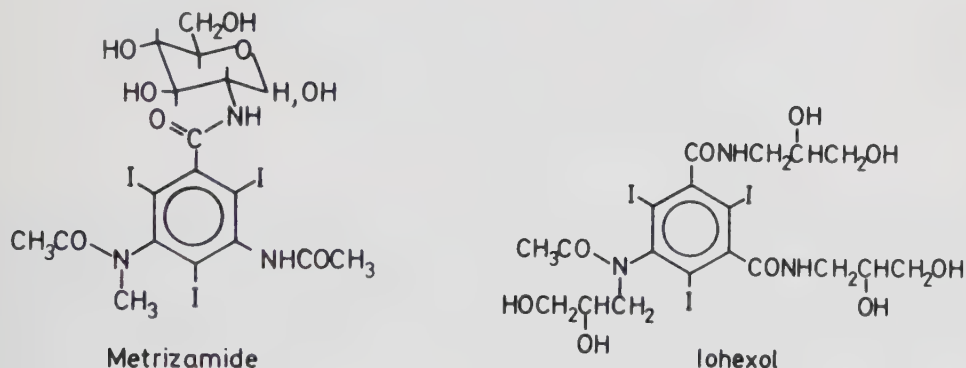


Fig. 1. Molecular structure of metrizamide and iohexol.

MATERIALS AND METHODS

Twenty-eight New Zealand White rabbits of both sexes weighing between 3.6 and 5.6 kg (mean 4.1 kg) were used for the study. The rabbits had free access to food and water prior to the investigation and the order of their use was statistically randomized. The animals were divided into 4 groups of 6 depending upon investigation time; 1, 2, 4 and 8 hours. There were another 4 rabbits who acted as controls receiving an artificial CSF medium instead of contrast medium; 2 were studied for one hour and 2 for 4 hours. All test animals had 0.5 ml of iohexol (180 mg I/ml) injected into the lumbar subarachnoid space. The artificial CSF medium (0.5 ml) used for the control animals was identical to that used by our group in previous metabolic studies /5/.

The animals were anesthetized with intravenous sodium pentobarbital during the entire experiment, supplemented with 1 % lidocaine for the surgical procedure.

The surgical preparation was identical to that previously described for the metrizamide study /6/. Initially a 5 F catheter was positioned in the abdominal aorta to allow monitoring of blood pressure, heart rate and arterial blood gases. The catheter was flushed with an isotonic solution containing 140 mM glucose, 70 mM Na⁺, 45 mM Cl⁻ and 25 mM CH₃COO⁻. The body temperature was also continuously monitored rectally using a thermistor probe. Following a laminectomy of the lumbar vertebrae 6 and 7 a polyethylene catheter (PE 10) was inserted into the subarachnoid space and placed with its tip at the level of L7 - S1. The site of insertion of the catheter through the dura was sealed with cyanoacrylate (Super glue). Before injection of the test solution the cranial end of the restraining board was elevated 30 - 35° to avoid gravitational cranial flow of the solution. The test solution was injected slowly over a 3 minute period while blood pressure and heart rate were closely monitored. Blood gases were monitored prior to and following injection. Upon completion of the injection the catheter was emptied of contrast medium by filling it with air after which it was sealed. Radiography was performed to verify the distribution of contrast medium.

At the end of the experiment the animals were sacrificed with an overdose of pentobarbital. A 0.2 ml sample of CSF was taken from the suboccipital area and another 0.2 ml CSF sample from the lumbar area for measurement of its iodine content. The spinal cord was then exposed and approximately 1 gram of the lumbar, thoracic and cervical cord samples were taken for iodine measurements. The tissue was briefly rinsed in saline to avoid surface contamination and weighed. The iodine content was analyzed with an X-ray excitation fluorescence spectrometer (XES) and expressed as mg I/g of tissue. The CSF samples were similarly analyzed.

RESULTS

All control measurements of blood gases, blood pressure, heart rate and body temperature were normal and constant during the experiment except for a slight initial drop in pO₂ in 2 animals (2 and 4 hour animals), a brief blood pressure and temperature drop in one animal (4 hour animal) and mild metabolic acidosis at the end of the experiment in one animal (8 hour animal). The control animals were all normal during the experiment.

The iodine content in the samples of the last 2 animals (2 and 4 hour animals) in the study could not be evaluated because of malfunction of the XES. In 6 animals it was not possible to withdraw a representative CSF sample from the lumbar region at the end of the study and these animals could not be used for evaluation of the relationship between lumbar-CSF and lumbar cord concentration. In one animal radiography showed contrast medium remaining in the tubing (Fig. 2, animal a) and in another animal there was extra-arachnoid deposition of a large portion of the contrast medium (Fig. 2, animal b). The first of these 2 animals (a) is probably displaced to the right in Fig. 2 as a result of a falsely high CSF concentration from the tube content. The other animal (b) should show a normal relationship between cord and CSF, although the initial concentration of contrast medium was lower than expected. However, this animal had to be excluded from further evaluation since nothing is known about the initial amount of subarachnoid contrast medium.

In a previous study we had shown a delay in disappearance rate of metrizamide from neural tissue with proportionally higher iodine concentration in the lumbar cord than CSF in the animals studied for 8 hours. This difference in CSF-tissue distribution was statistically significant at the one and 8 hour animals when tested with the Bonferroni method of multiple comparisons /6/. In spite of no indication for such a relationship with iohexol (Fig. 2) we also tested the iohexol animals with the Bonferroni method for each time period. There was no significant difference between any of the groups.

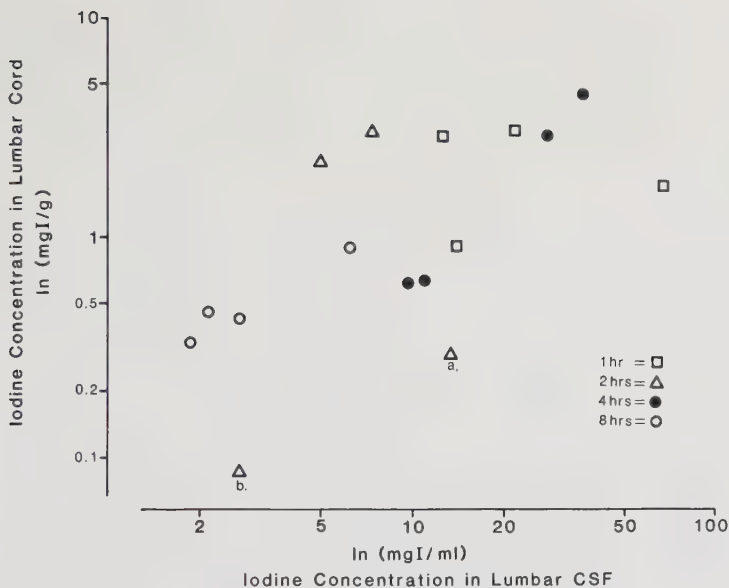


Fig. 2. Iohexol - the iodine concentration in the lumbar cord is plotted against the concentration in the lumbar cerebrospinal fluid. Data points marked "a" or "b" are discussed in the text.

In conformity with the previous metrizamide study there is a decline in the lumbar CSF concentration with time as expected (Fig. 3). There is, however, a difference in the suboccipital CSF findings. In this study we could not detect any increase in contrast medium concentration with time and the concentration, (with two exceptions) was always below 0.06 mg I/ml (Fig. 3). The reason for this apparent difference in cranial transport of the contrast medium is hard to understand. The number of animals in each study is too few to state whether this is a statistically significant finding.

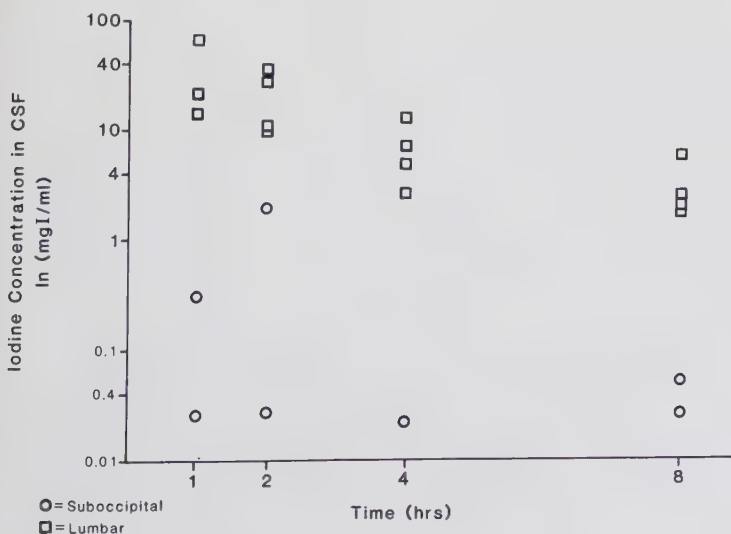


Fig. 3. Iohexol - the iodine concentration in lumbar cord versus time. Animals which had the upper extension of contrast medium above or below the usual L1 - L2 level are indicated with symbols.

The relationship between the lumbar cord concentration with time is somewhat different for iohexol (Fig. 4) compared to the previous metrizamide study. In spite of a large spread there is a definite downward trend with time for the iohexol animals whereas metrizamide demonstrated a more or less constant concentration between one and 8 hours. The decrease in concentration with time for lumbar CSF as well as lumbar cord are consistent with a simple diffusion relationship for iohexol.

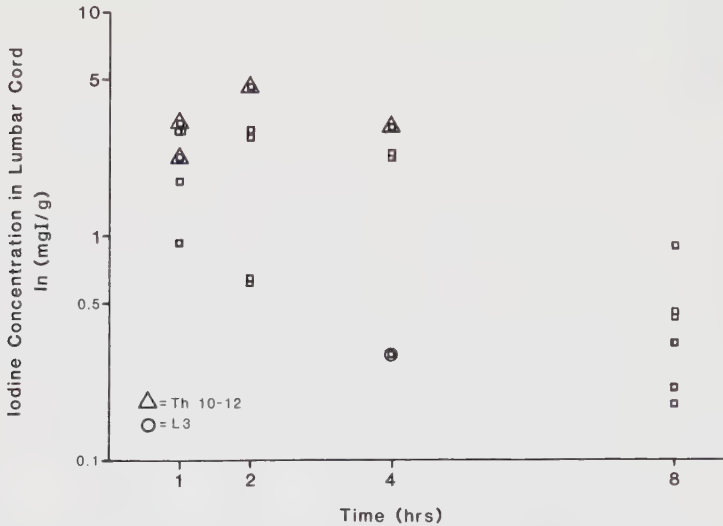


Fig. 4. Iohexol - the iodine concentration in lumbar cord versus time. Animals which had the upper extension of contrast medium above or below the usual L1 - L2 level are indicated with symbols.

The decrease in mean iodine concentration in the lumbar cord is also demonstrated in Table I, in which can also be seen that the cranial flow of CSF is extremely low. The thoracic and cervical cord contrast medium concentrations are significantly lower than that of the lumbar cord for all time periods ($p < 0.05$ according to Student's t-test for paired samples). As for metrizamide, iohexol always had a lower concentration in the thoracic and cervical cord than in the lumbar cord. The percent difference in concentration is highly significant ($p < 0.001$) for each group (Student's t-test for paired samples) (Table II). The small decrease in percent difference over time for each group is further evidence of low cranial flow of CSF. The major drainage in immobilized rabbits must take place in the lumbosacral region following lumbar myelography.

Evaluation of the radiographic examinations following contrast medium injection demonstrated some inter-animal variation in the size of the dural sac. As a result, there was some variability in the level that the contrast medium reached initially in the spinal canal. In most cases the cranial portion of the contrast medium column was at the level of L1 - L2 vertebrae but in 4 animals it was as high as Th10 - Th12 and in one animal it only reached L3. Such a difference in dural sac volume and the subsequent extension of the contrast medium column could influence the contrast medium concentration of the lumbar cord since the contrast medium clear-

TABLE I. Mean iodine concentration (mg I/g tissue) in the spinal cord at 1, 2, 4 and 8 hours after administration of iohexol into CSF.

Sample	1 hour	2 hours	4 hours	8 hours
Lumbar cord	2.33 ± 0.35 SEM	2.33 ± 0.76 SEM	1.99 ± 0.59 SEM	0.42 ± 0.11 SEM
Thoracic cord	0.30 ± 0.10 SEM	0.32 ± 0.29 SEM	0.13 ± 0.07 SEM	0.07 ± 0.03 SEM
Cervical cord	0.039 ± 0.013 SEM	0.024 ± 0.018 SEM	0.022 ± 0.010 SEM	0.030 ± 0.011 SEM
No. animals	6	5	4	6

TABLE II. Intra-animal difference in concentration of iohexol between the three parts of the spinal cord.

Time	D i f f e r e n c e s , %		No. animals
	$(\frac{Th}{L} - 1) \times 100$	$(\frac{Cerv}{L} - 1) \times 100$	
1 hour	-86.4 ± 4.6 SEM	-98.0 ± 1.0 SEM	6
2 hours	-92.9 ± 6.3 SEM	-99.4 ± 0.4 SEM	5
4 hours	-90.4 ± 4.3 SEM	-99.0 ± 0.5 SEM	4
8 hours	-84.3 ± 3.2 SEM	-91.9 ± 3.4 SEM	6

ance seems to occur in the lumbo-sacral region. The more cranial the extension of the contrast medium column, the longer the contact time for the sampled mid-lumbar cord assuming CSF drainage rate is identical for all animals. The number of animals in this study is too small to evaluate the significance of this observation. However, a comparison of the 4 animals with the upper contrast medium level at Th10 - Th12 to those at L1 - L2 and the one at L3 (Fig. 4) indicates that there may be some differences which could influence the spread of data within each group.

DISCUSSION

The central nervous system is dependent upon a stable extracellular environment and on continuous supply of energy for its normal function. To achieve this the CNS relies both upon the blood-brain barrier to hinder the entrance of unwanted substances as well as a well-regulated selective transport mechanism for required solutes such as glucose. This barrier efficiently blocks the passage of most water-soluble and electrically charged solutes like ionic contrast media. There are, however, a few small areas where this barrier is missing and small amounts of barrier impermeable solutes may pass. Intravascular contrast media can thus normally be traced in CSF within the first few hours following injection but the total amount demonstrated in CSF is within 1 - 2 % of the total volume injected in animals with normal kidney function /12/.

By means of the blood-brain barrier the brain is thus normally protected from the toxic effects of conventional ionic contrast media. The exact role of CSF is as yet unknown but it probably serves as a supplier of nutrients, hormones and other messenger substances as well as a drainage medium for waste products and a buffer for extracellular fluid. To achieve this there is no barrier between the CSF and the extracellular space of the CNS with the exception of a few areas such as the choroid plexus. Water-soluble contrast media can therefore diffuse from the CSF into the ECS of the CNS. In spite of their superiority as contrast agents in achieving a detailed delineation of the subarachnoid space, the ionic structure of conventional vascular contrast media makes them far too toxic to be used in this region. The development of the first non-ionic contrast medium, metrizamide, was a major achievement in reducing neurotoxicity, but some problems still remained. The cause of the toxicity from metrizamide has not yet been fully investigated but most experiments have been related to its 2-DG-component.

Various studies have demonstrated the possibility of metabolic disturbances as a result of competition between D-glucose and the 2-DG portion of metrizamide. This could take place extracellularly at the specific glucose membrane carrier level /5/ or intracellularly at the enzymatic level /3/. Experiments by Fenstermacher et coll /8/ and by our group /6/ have indicated some trapping of metrizamide in neural tissue. This could occur as a result of intracellular transport or extracellular binding, but no studies so far have been able to demonstrate intracellular transport of metrizamide in neural tissue /9, 10/.

The idea of binding of the 2-DG component to the specific glucose membrane carrier is in many ways attractive. If such a binding is the cause of the delayed excretion of metrizamide other non-ionic contrast media, like iohexol, should not show the same pattern. In the present study lumbar CSF and cord concentrations of iohexol decrease in an approximately parallel fashion with time and there was no sign of neural tissue binding of iohexol in the rabbit following myelography. Iohexol shows a simple diffusion pattern and unlike metrizamide /6/ we were unable to show any different regression lines for any time sequence.

Since most of the serious neurologic side effects are a result of contrast medium affecting brain tissue it is also important to evaluate the transport of CSF and its drainage to the blood. The previous metrizamide study showed a small and very slow cranial transportation of CSF /6/. In the present study the cranial transport seems even less pronounced but there is no significant difference between the two. Most of the lumbar subarachnoid contrast medium in this experimental model is drained via a lumbo-sacral route.

These experiments appear to indicate that metrizamide and iohexol have different distribution kinetics in rabbit neural tissue. The iohexol distribution appears to follow the simple diffusion model whereas the metrizamide distribution indicates binding or uptake. Metabolic experiments comparing the two also demonstrated that iohexol does not interfere with neural tissue metabolism /7/ unlike metrizamide which does /5/. Preliminary data from clinical trials /2, 13/ and electrophysiologic experiments /4/ with iohexol have been very encouraging. The data from our present study and previous metabolic experiments further support that iohexol is less neurotoxic than metrizamide and therefore should be a better myelographic contrast medium.

REFERENCES

1. AMUNDSEN P.: Metrizamide in cervical myelography: Survey and present state. *Acta Radiol Suppl* 355 (1977), 85 - 97
2. BATTY V.B.: Cervical myelography using iohexol (Omnipaque), a new contrast medium. *Clin Radiol* 35 (1984), 75 - 77
3. BERTONI J.M., SCHWARTZMAN R.J., vanHORN G. and PARTIN J.: Asterixis and encephalopathy following metrizamide myelography: Investigations into possible mechanisms and review of the literature. *Ann Neurol* 9 (1981), 366 - 370
4. BRYAN R.N., CENTENO R.S., HERSHKOWITZ N., POELSTRA R.J. and OSATO M.S.: Neurotoxicity of iohexol: A new nonionic contrast medium. *Radiology* 145 (1982), 379 - 382
5. EKHOLM S.E., REECE K., COLEMAN J.R., KIDO D.K. and FISCHER H.W.: Metrizamide - a potential in vivo inhibitor of glucose metabolism. *Radiology* 147 (1983), 119 - 121
6. EKHOLM S.E., FOLEY M., KIDO D.K. and MORRIS T.W.: Metrizamide lumbar myelography in rabbits - A study of contrast media penetration and resorption. *Acc for publ in Acta Radiol Diagnosis* (1984)
7. EKHOLM S.E., REECE K., FOLEY M., KIDO D.K. and MORRIS T.W.: The effect of iohexol on glucose metabolism compared to metrizamide. *Invest Radiol* (1984) In press
8. FENSTERMACHER J.D., BRADBURY M.W.B., duBOULAY G., KENDALL B.E. and RADU E.W.: The distribution of ^{125}I -metrizamide and ^{125}I -diatrizoate between blood, brain and cerebrospinal fluid in the rabbit. *Neuroradiol* 19 (1980), 171 - 180
9. GJEDDE A.: The blood-brain barrier is impermeable to metrizamide. *Acta Neurol Scand* 66 (1982), 392 - 395
10. GOLMAN K.: Distribution and retention of ^{125}I -labelled metrizamide after intravenous and suboccipital injection in rabbit, rat and cat. *Acta Radiol Suppl* 335 (1973), 300 - 311
11. HAMMER B.: The pathophysiology of intrathecally injected contrast media. Short communication in: Felix R., Kazner E. and Wegener O.H. (Eds): *Contrast Media in Computed Tomography*. Amsterdam - Oxford - Princeton: Excerpta Medica (1981), 52 - 57
12. JUNCK L. and MARSHALL W.H.: Neurotoxicity of radiological contrast agents. *Ann Neurol* 13 (1983), 469 - 484
13. LOSSIUS R., ELDEVIK O.P., WEBER H., OFTEDAL S.I. and STRÖMME J.H.: First clinical trial with iohexol in myelography. *Acta Radiol Diagnosis* 24 (1983), 499 - 502
14. SAGE M.R., WILCOX J., EVILL C.A. and BENNESS G.T.: Brain parenchyma penetration by intrathecal ionic and nonionic contrast media. *AJNR* 3 (1982), 481 - 483

IOHEXOL AND METRIZAMIDE LUMBAR MYELOGRAPHY
A DOUBLE-BLIND CLINICAL TRIAL

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ABSTRACT

Two non-ionic contrast agents, iohexol and metrizamide, were compared in a double-blind clinical trial which included 50 patients who underwent lumbar myelography for disc herniation or spinal stenosis. The frequency of adverse reactions was lower for iohexol which is recommended for extended trials and for examination of other compartments of the subarachnoid space.

INTRODUCTION

The non-ionic contrast medium, iohexol (Omnipaque^R), has been used in the vascular space for a few years and has been shown to have almost no pharmacological effects and a lower frequency of subjective reactions when compared to the currently used ionic contrast media /1, 2, 9, 10/. Intrathecal iohexol induces significantly less neurotoxic reactions than metrizamide (Amipaque^R) in laboratory animals /5, 13/ and does not cause adhesive arachnoiditis in monkeys /7/. The results from clinical trials have been encouraging /3, 6/. The objective of this study was to compare iohexol and metrizamide in lumbar myelography under controlled clinical conditions.

MATERIALS AND METHODS

The study consisted of 50 patients who all had symptoms and signs consistent with herniated lumbar disc or spinal stenosis. The patients, 18 years or older, entered the study consecutively provided they did not fit into one of the exclusion criteria listed in Table I. Twenty-five patients received iohexol (12 males and 13 females; mean age 47.5 years, range 18 - 79) and the other 25 received metrizamide (15 males and 10 females; mean age 42.6 years, range 18 - 64). The contrast medium assigned to each patient was determined by a computer generated randomized code. Only the assistant who prepared the contrast medium for injection had access to the code. The patients were premedicated with 0.5 mg atropine and 100 mg nembutal. The puncture was performed with a 20 gauge needle with the patient in the prone or sitting position. After removal of 5 cc of cerebrospinal fluid (CSF) for analysis a standard lumbar myelography was performed with the patient in the prone position. At the end of the examination the patient was turned into the supine position, with raised head, to obtain an ap view of the conus region. The contrast medium was not given above the 10th thoracic vertebra. Both contrast media were administered in a concentration of 180 mg I/ml. The mean dose was 16.12 ml (range 11 - 17 ml) for iohexol and 16.38 ml (range 12 - 17) for metrizamide.

Post-procedural care included helping the patient into the upright position for 30 - 60 s directly after termination of the myelography in order to facilitate collection of the contrast medium in the caudal dural sac. After returning to their rooms, rest was instituted for 24 hours with the head of the bed raised 30°. Rest-room privileges were allowed 8 hours after the examination. All patients had an extensive neurological examination before, 4 - 6 hours and 22 - 24 hours after the myelography. The examination included tests for mental status, concentration, orientation, memory, cranial nerve function, coordination, arm and leg reflexes and sensibility with special attention paid to perianal and perigenital regions. The examinations were performed by 2 trained neurosurgeons. Routine blood analyses, including electrolytes, liver enzymes and hematological tests were performed before and 24 hours after each myelography.

TABLE I. Exclusion criteria for participation in double-blind trial.

Patients with known or suspected hypersensitivity to iodine-containing agents

Women of child bearing potential when the degree of risk is greater than the potential benefit of the procedure

Pregnant or lactating women

Patients having a previous intra-thecal or subarachnoid puncture within 48 hours

Patients who are receiving any other investigational drug

Emergency case

Patients with grossly bloody tap

Out-patients

Patients who have received any drugs intra-theccally or epidurally preceeding one (1) month

Patients who have had spinal cord surgery in the preceeding one (1) month

Patients receiving neuroleptic drugs

Patients with known or suspected decreased seizure threshold

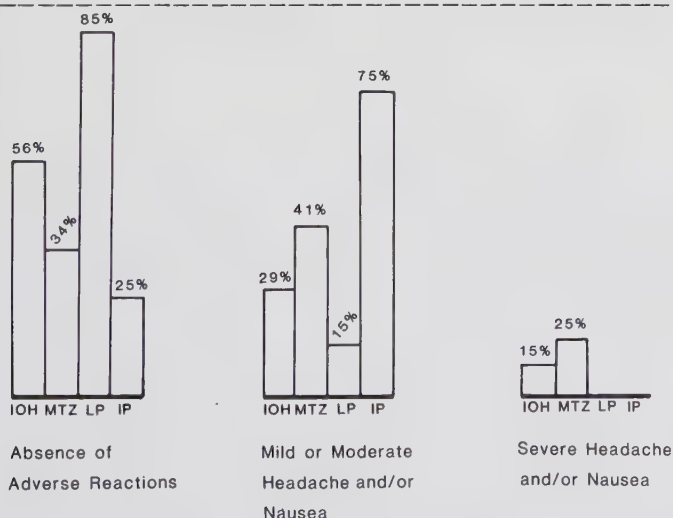


Fig. 1. Frequencies of adverse reactions in percent reported after "general" questions. IOH = iohexol myelography (n = 25), MTZ = metrizamide myelography (n = 25), LP = diagnostic lumbar puncture (n = 20), IP = iophendylate myelography (n = 20).

In order to evaluate the incidence of adverse reactions the patients were seen in their ward twice during the afternoon of the day of examination, the day after the examination and, in case of persistent symptoms, during the succeeding days until the symptoms disappeared. Expected symptoms were not specifically elicited. Instead questions like "how do you feel"?, "any problems"?, etc. were posed and when symptoms were reported the patients were asked to characterize them as mild, moderate or severe.

For comparison 2 other groups were also questioned for adverse reactions in the same way. One of these groups comprised 20 patients (10 males and 10 females) with symptoms suggesting a cervical disc herniation. These patients had the same preparation and the same post-myelographic regimen as the patients who had lumbar myelography except that they received iophendylate (Pantopaque^R). The second group consisted of another 20 patients (10 males and 10 females) who for various reasons had a diagnostic lumbar puncture as part of a neurological work-up. These 2 groups of patients were not subjected to other tests used for the lumbar myelography study groups.

An independent radiologist assessed the image quality. The data was graded on a 1 to 4 scale:

grade 1	non-visualization of the subarachnoid space;
grade 2	poor visualization of the subarachnoid space with insufficient information to make a radiological diagnosis;
grade 3	good visualization of the subarachnoid space with sufficient information to make a radiological diagnosis;
grade 4	excellent visualization of the subarachnoid space with excessive information to make a radiological diagnosis.

RESULTS

Adverse reactions

The subjective adverse reactions reported by the patients are summarized in Fig. 1. As expected the most commonly reported adverse reaction was headache. This symptom appeared in 36 % (9 cases) of the iohexol group and in 62.5 % of the metrizamide group (15 cases). The corresponding adverse reactions for patients with iophendylate myelography was 75 % and for diagnostic lumbar puncture 15 %. Four patients who received metrizamide and 5 who received iohexol complained of nausea. Nausea always appeared in combination with headache and was combined with vomiting in a few instances. A headache not relieved by analgesic drugs and with a duration exceeding 24 hours was recorded in one patient who had metrizamide and in 2 patients who had iohexol. One of the latter patients had both headache and nausea for 3 days following the myelography. Aggravation of pre-existing back pain or sciatica occurred in 2 patients following iohexol myelography. Both of these patients characterized the symptom as being "severe". The pain disappeared 12 and 24 hours after myelography, respectively. One patient who had iohexol reported an episode of dizziness which lasted 5 - 10 minutes. No convulsive reactions or allergic reactions were recorded.

Neurologic examination

Neurologic and physical findings referable to the lumbar region were routinely present at the pre-myelographic examination. However, no change was recorded in any patient at repeat examinations 4 - 6 hours and 22 - 24 hours after myelography. No change in mental state or mental function was recorded in any of the patients at the same time intervals.

Routine Laboratory Tests and Vital Signs

The results of routine analyses of blood samples obtained before and after myelography did not demonstrate any significant change in the parameters studied. Routine analysis of CSF specimens was unremarkable with the exception, in a few cases, of red blood cells derived from the puncture. No significant changes were recorded in blood pressure, pulse rate or body temperature.

Radiographic information

Visualization of the cauda equina was judged as poor in 5 metrizamide patients and one iohexol patient. The difference was, however, due either to technical factors (4 patients) or obstruction of the canal by a large disc herniation (1 patient). The visualization of nerve roots and nerve root sleeves was generally considered to be excellent with both media.

DISCUSSION

Most of the variation in the frequency of adverse reactions after myelography with metrizamide (66 %) and iohexol (44 %) is accounted for by the symptoms of headache and nausea which were more common among the metrizamide patients (62.5 % versus 36 %). However, 4 patients who had iohexol experienced symptoms which should be discussed. Two patients had rather protracted pain in both legs and 2 had headache which they characterized as severe. Increase in pre-existing leg pain is a well-known complication of myelography in patients with lumbar disc herniation /8, 14/ and may be caused by unusual positioning of the patient during the examination. One patient who complained of leg pain for 24 hours post myelography had a marked L3 - L4 disc bulge combined with spinal stenosis. The other patient, who had a right-sided disc herniation at the level of L4 - L5, experienced an abrupt increase in bilateral leg pain when turned from prone to supine position suggesting compression of the cauda equina. One of the patients with severe headache experienced aggravation of the headache in the upright position suggesting CSF leakage. The other patient with severe headache was later thought to have emotional problems. This patient had a long history of various ailments and requested more analgesic drug than the average patient. In addition to the severe headache, he also complained of nausea and a feeling of uneasiness.

Headache was frequently experienced in the iophendylate group (75 %). However, the frequency of iophendylate headaches cannot be directly compared to those experienced by patients undergoing iohexol or metrizamide myelography. The injection of iophendylate was performed with an 18 gauge needle and moreover the selection of patients for cervical iophendylate myelography and lumbar myelography was different. For practical reasons it was not possible to examine the adverse reactions associated with iophendylate under blind conditions, nor was it for the diagnostic lumbar puncture group. However, it is our belief that subjective adverse reactions from different agents or procedures can only be compared with any degree of accuracy under the conditions offered by a double-blind trial where the patient population is relatively homogeneous, the patients are prepared in a standardized manner,

and physical examination and after-care are standardized. Frequencies of adverse reactions reported after specific questions for single symptoms should not be compared to frequencies reported after general questions. The remarkably low frequency of headache in the diagnostic lumbar puncture group may illustrate the truth of this statement - 15 % incidence of headaches in the diagnostic lumbar puncture group after casual questions as opposed to 36 % in a group of healthy student subjected to diagnostic lumbar puncture and questioned specifically for this symptom /17/.

Clinical examination did not disclose any obvious change in mental status in either the iohexol or metrizamide patients. Short-term mental deterioration, in extreme cases characterized as a "psycho-organic syndrome", has been reported by several workers as a complication of metrizamide myelography /4, 11, 12, 15,16/. However, this type of reaction is unusual with the dosage used in this investigation. Evaluation of possible psychic reactions from iohexol must await extended trials.

Routine laboratory data of blood specimens and recordings of vital signs are easily obtained. In general, these parameters are not relevant for the assessment of toxicity of modern contrast media administered in the subarachnoid space /3,6/. As expected, there were no differences in the 2 non-ionic media under trial.

The diagnostic potential as shown by visualization of the detailed anatomy was estimated to be equal with both contrast media. This is also in keeping with the common belief that media with small differences in physical characteristics and iodine content provide equal opacification and delineate intra-theal structures with equal accuracy.

CONCLUSION

The high tolerance of the CNS to iohexol as shown by experimental work and from earlier open phase 2 studies is recognized. When compared to metrizamide under blind conditions, iohexol turned out to be superior to metrizamide with respect to adverse reactions. Iohexol also has the advantage of being dispensed in a stable solution. The medium can be recommended for extended trials including examination of other compartments of the subarachnoid space. In future studies close attention should be paid to mental function, convulsive reactions and possible long-term effects such as adhesive arachnoiditis.

REFERENCES

1. AHLGREN P.: Iohexol compared to urografin meglumine in cerebral angiography. A randomized double-blind cross-over study. *Neuroradiol* 23(1982), 195 - 198
2. AMUNDSEN P., DUGSTAD G., PRESTHUS J., AHLGREN P., CRONQVIST S., HOLTÅS S., SKALPE I., LUNDERVOLD A., DAHLSTRÖM K and RENAA T.: Angiography with iohexol: A multicentre clinical trial. *Acta Radiol Diagnosis* 23 (1982), 529 - 534
3. ERICSON K., HINDMARSH T. and HANNERZ J.: Experience with iohexol in lumbar myelography. *Acta Radiol Diagnosis* 24 (1983), 503 - 505
4. GELMERS H.J.: Acute psycho-organic reactions after cervical myelography using metrizamide. *Clin Neurol Neurosurg* 83 (1981), 57 - 61
5. GOLMAN K., OLIVECRONA H., GUSTAFSON C., SALVESEN S., ALMÉN T. and MALY P.: Excitation and depression of non-anesthetized rabbits following injections of contrast media into the subarachnoid space. *Acta Radiol Diagnosis Suppl* 362 (1980), 83 - 86
6. GRÖNNERÖD T.A., ELDEVIK O.P., HINDMARSH T., KENDALL B. and NAKSTAD P.H.: Documentation of a new contrast medium for the subarachnoid space. Demands, design and results from the first multicentre trial with iohexol. *Acta Radiol Diagnosis* 24 (1983), 487 - 491
7. HAUGHTON V.M.: The effect of myelographic contrast media on the arachnoid. In: Amiel (Ed): *Contrast Media in Radiology*, Berlin, Heidelberg, New York, Springer Verlag, (1982), 149 - 153
8. HINDMARSH T.: Lumbar myelography with meglumine iocarmate and metrizamide. *Acta Radiol Diagnosis* 16 (1975), 209 - 222
9. HINDMARSH T., BERGSTRAND G., ERICSON K. and OLIVECRONA H.: Comparative double-blind investigation of meglumine metrizoate, metrizamide and iohexol in carotid angiography. *AJNR* 4 (1983), 347 - 349
10. LEVORSTAD K., KOLBENSTVEDT A., SOMMERFELT S., ZACHRISSON B.E., JAGENBURG R., EGEBLAD M., NIELSEN N.T., SJÖBERG S., OLDBRING J. and SVEEN K.: Tolerability and diagnostic usefulness of iohexol in human urography: An open multicentre clinical trial. *Acta Radiol Diagnosis* 23 (1982), 491 - 496
11. MINDUS P. and HINDMARSH T.: Mental reactions after subarachnoidal administration of Amipaque (metrizamide). *Läkartidningen* (Summary in English) 79 (1982), 3206 - 3208
12. SCHMIDT R.C.: Mental disorders after myelography with metrizamide and other water-soluble contrast media. *Neuroradiol* 19 (1980), 153 - 157
13. SIEFERT H.M., PRESS W.R. and SPECK U.: Tolerance to iohexol after intracisternal, intracerebral and intraarterial injection in the rat. *Acta Radiol Diagnosis Suppl* 362 (1980), 77 - 81

14. SKALPE I.O., TORBERGSEN T., AMUNDSEN P. and PRESTHUS J.: Lumbar myelography with metrizamide. *Acta Radiol Diagnosis Suppl* 335 (1973), 367 - 379
15. SMITH M.S. and LAGUNA J.F.: Confusion, dysphasia and asterixis following metrizamide myelography. *Can J Neurol Sci* 7 (1980), 309 - 311
16. SORTLAND O., LUNDERVOLD A. and NESBAKKEN R.: Mental confusion and epileptic seizures following cervical myelography with metrizamide. Report of a case. *Acta Radiol Diagnosis Suppl* 355 (1977), 403 - 406
17. TOURTELLOTTOE W.W., HAERER A.F., HELLER G.L. and SOMERS J.E.: Post-lumbar puncture headaches. Charles C Thomas, Springfield, IL, USA (1964)



